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SCIENTIFIC AGRICULTURE

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MOISTURE DETERMINATION OF SILAGE, HAY AND GRAIN

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Modern methods of harvesting silage, hay and grain require an accurate estimation of the moisture content of the crop. This is essential to allow efficient use of the field machinery and to ensure that the crop is stored in the proper condition. The method of moisture determination must be rapid to be of value. Tests have shown that green hay in the field will lose as much as 10 per cent of moisture per hour on a good curing day. It is possible, therefore, that a precise moisture determination requiring more than half an hour could introduce an error of 5 per cent or more in the calculation of the moisture content of the material in the field. With silage or hay such an error could produce feed of inferior quality, and with grain the moisture must also be accurately estimated to avoid losses.

A rapid moisture tester, to be of real value to a farmer, must be portable, simple to operate and provide a convenient means of determining the condition of the crop in the field.

Several methods which vary in accuracy and suitability have been developed. An early method using calcium carbide to drive off the moisture was described by Parks (1) and was later developed for sale as a commercial unit. Another method that pressed the sample to determine the moisture was reported by Woodward (2). Recently Dexter (2, 4, 5, 6) has developed methods that are based on the relative humidity of the air surrounding the sample as well as other methods employing various means of applying heat to remove the moisture from the sample. Also discussed in this paper are simple methods using heated air to drive the moisture from the sample.

In conducting tests on the rapid methods of moisture determinations, it was necessary to have a suitable one with which to compare the results of the experiments. The method used as a standard during the tests utilized an electric laboratory oven. The samples were placed in the oven at a controlled temperature of 103° C. for a period of 8 hours or more. At the end of this time they were removed and placed in a desiccator to cool. The loss in weight was used to determine the moisture content of the samples. The oven method, although accurate, is of no practical value to the field operator since the time required for the determination makes the results obtained of no use to him.

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METHODS

The methods of rapidly determining the moisture content of silage, hay and grain tested at the Central Experimental Farm, Ottawa, are as follows:

Experiment 1—Calcium Carbide Method (1)

Description of Equipment

The apparatus used in this test consisted of the following: accurate scales, reading in grams; a supply of powdered calcium carbide and several seamless tins of approximately one pint capacity. However, a complete commercial kit can be obtained which may be used to determine the moisture content of various crops by the same method.

Procedure

Thirty grams of the sample and 100 grams of carbide are placed in the tin and shaken together. The tin is then allowed to cool and the contents agitated again. This is continued until heating no longer occurs. The loss in weight is then determined and a chart (1) used to obtain the percentage of moisture in the sample. When the commercial kit is used the scales read directly in percentage of moisture and no calculations are necessary.

TABLE 1.—RESULTS OF THE CALCIUM CARBIDE METHOD

(Data in per cent moisture)

Sample	Crop	Carbide Method			Oven Method
		Test #1	Test #2	Av.	
1	Alfalfa and clover	86.0	Ignited	86.0	84.5
2	"	Ignited	86.5	86.5	83.4
3	"	78.0	79.5	78.8	80.0
4	"	69.5	70.5	70.0	69.6
5	"	56.0	57.0	56.5	62.7
6	"	40.0	38.5	39.3	47.0
7	"	26.5	26.5	26.5	31.1
8	"	18.0	17.0	17.5	20.3
9	"	14.0	17.0	15.5	16.2

Discussion

While this method is relatively rapid, requiring approximately half an hour per sample, it is not accurate for all moisture contents. When the moisture content of grass is 75 per cent or more the heat of the reaction may ignite the sample, unless care it taken to allow the heat to escape readily or unless the speed of the reaction is reduced by judicious management of the carbide.

Dry samples below 20 per cent are often inaccurately determined. Between these limits an accuracy of $\pm$ 3 per cent can be obtained if care is taken to allow the reaction to reach completion. Samples 5, 6, and 7 are examples of this error. It can be seen from the table that the two tests checked rather well but the error is obvious when compared with the oven method. These samples were made up of rather coarse stems which retained the moisture and may have caused the error in this determination. With careful operation this method will determine the moisture content of samples ranging from 20 per cent to 75 per cent moisture with a variation of less than $\pm$ 3 per cent.

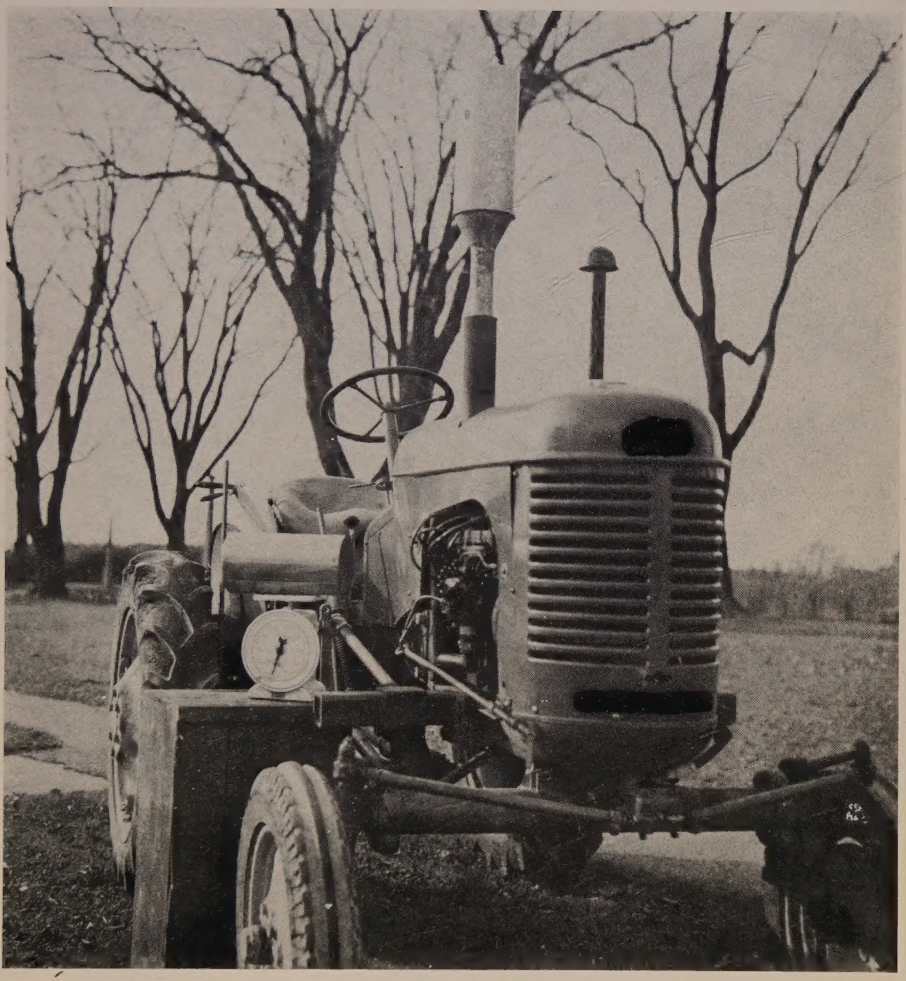

FIGURE 1. Exhaust oven in position on the tractor with the sleeve and sample on the scale.

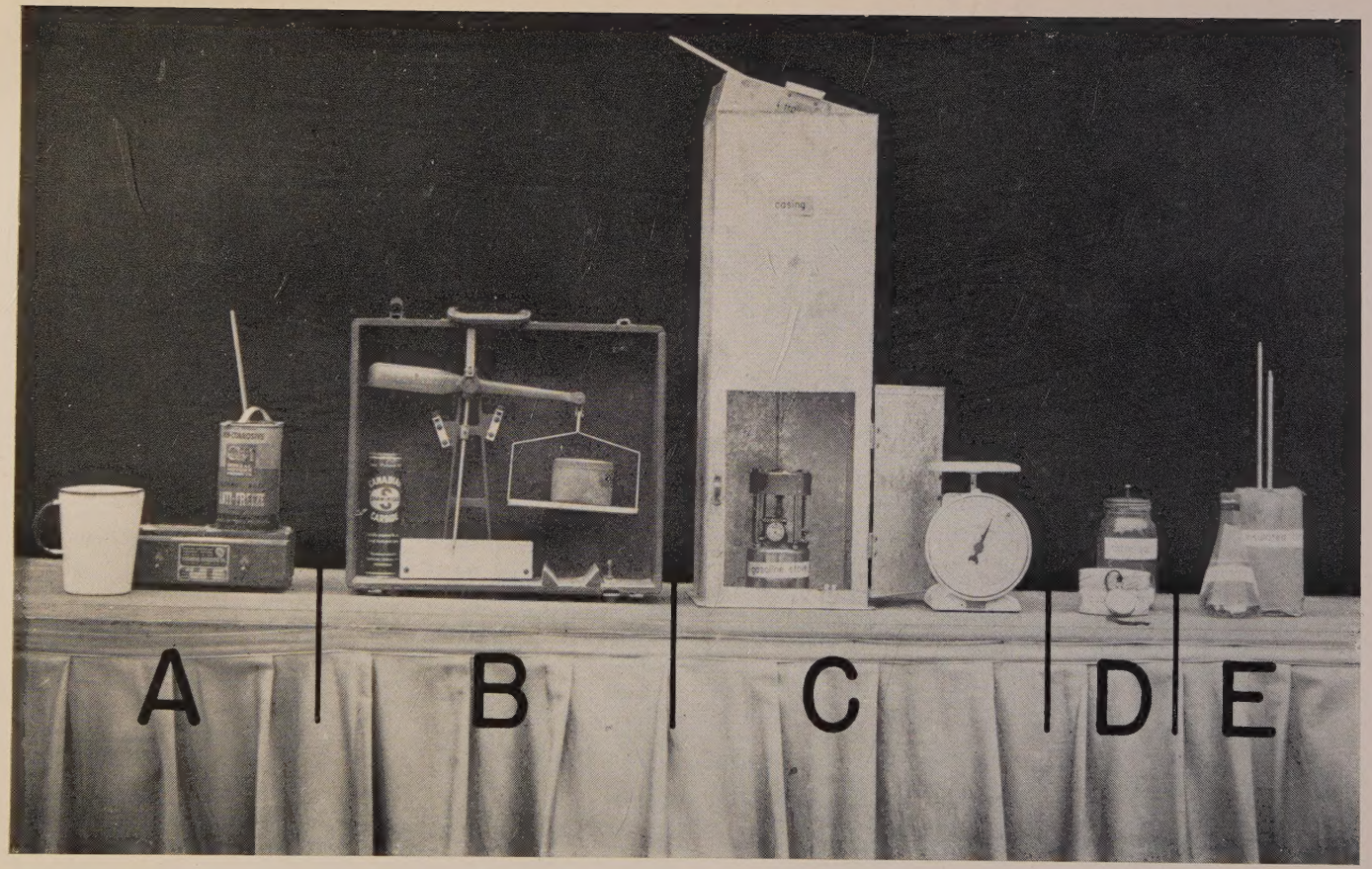

FIGURE 2. Equipment required for five methods of moisture determination.

A.—Oil Immersion Method (including scale from C)
B.—Calcium Carbide Kit
C.—Tray Drier Method
D.—Common Salt Method
E.—Relative Humidity Method

Experiment II—Pressure Method (2)

Description of Equipment

A perforated 2-inch pipe, 12 inches long, is fitted with a plunger. By means of levers and weights a given force may be applied to the plunger.

Procedure

The sample is chopped and placed in the pipe to a depth of 3 to 6 inches. A weight determined by the type of crop is placed on the lever and is allowed to remain in place for one minute. If the juice is expressed from the holes in the pipe the moisture content is above approximately 68 per cent. If no moisture appears, the sample is claimed to be less than 68 per cent moisture (2).

Discussion

Estimates of the moisture content can be made only with materials ranging from 60 per cent to 75 per cent moisture. This test requires approximately half an hour including the time to prepare the sample. While it was found that some indication of the moisture content could be obtained, the kind of crop material in the sample, length of cut and method of chopping caused such a wide variation that the results were of little practical value.

Experiment III—Gasoline Engine, Exhaust Oven Method (3)

Description of Equipment

A metal tube, 4 or 5 inches in diameter and about a foot long, is attached to the end of the exhaust pipe of a gasoline engine. This chamber is used as the oven in which the sample is dried. To avoid loss of the sample, a sleeve slightly smaller than the oven having screens in each end holds the material in place. For these experimental tests a 200° C. thermometer is used to indicate the temperature at the centre of the sample. A 500-gram spring scale with an adjustable face is employed to weigh the sample.

Procedure

To obtain the weight of the sample the sleeve is placed on the scales, the face of the scale is adjusted to zero, the sample is inserted into the sleeve, and the weight of the sample is recorded. With the engine operating and the "oven" in position on the exhaust pipe, the sample is introduced into the oven. The sample is reversed, end for end, in the oven each minute, weighed every two minutes and the loss in weight recorded. When there is no further loss in weight, the sample is considered to be dry and the percentage of moisture is calculated. To prevent charring, the temperature of the sample should not be allowed to rise above 140° C. at the centre of the sleeve. The temperature is regulated by the speed of the engine.

Discussion

Table 2 shows that a wide range of moisture contents can be determined with considerable accuracy. The large error in sample number 4 was due to a slight charring of the material. The time taken for the tests varied from 10–45 minutes. This variation is directly in proportion to the amount of moisture in the sample. It should be noted that samples

TABLE 2.—RESULTS OF TESTS ON EXHAUST "OVEN" METHOD

Sample No.	Initial Weight of Sample	Final Weight of Sample	% Moisture		Time for Test
			Exhaust Oven	Electric Oven	
	grams	grams			minutes
1	216	54	75.0	74.6	35
2	244	65	73.4	72.0	45
3	184	65	64.7	65.4	40
4	128	56.6	55.8	52.2	25
5	164	91	44.5	44.2	25
6	147	94	36.0	34.1	20
7	153	107	30.0	28.9	20
8	108	87	19.4	17.4	15
9	116	99	14.6	13.5	10

2 and 3 took longer than sample 1. This can be attributed to the inexperience of the operator since samples 2 and 3 were tested before the operator was accustomed to the equipment. Also since sample 2 is rather large, the circulation of the hot air was restricted and reduced the efficiency of the unit. When care was taken not to char the sample, the result compared favourably with the laboratory method.

Experiment IV—Tray Dryer Method

Equipment

A casing 30 inches high and 12 inches in diameter is used to contain a gasoline heater and support a wire sample basket. To enable the regulation of the heat applied to the sample, an adjustable door is placed near the bottom of the casing. Accurate scales reading in grams and a 200° C. thermometer are also necessary. Heat is supplied by the gas-burning camp-stove.

Procedure

100 grams of chopped material or grain are placed in the wire tray and set on the casing above the gasoline heater. The temperature is increased to 145° C. for hay or 160° C. for grain and maintained by adjusting the casing door. After about 5 minutes the sample is weighed and the tray returned to the casing. This is repeated at two-minute intervals until no loss in weight is noted. The moisture content is then calculated by subtracting the final weight from 100. To ensure uniform drying, bulky materials such as dry hay should have the sample reduced to 50 grams.

Discussion

From the data in Table 3 it can be seen that this method is sufficiently accurate to be used to guide field operations. The variation in time required depends upon the nature of the sample. Materials with coarse stems, a compact structure and high moisture content require the greatest length of time. Test No. 7 needed 19 minutes because the sample was compact. This caused poor air circulation and required a reduction in temperature to prevent the sample from being charred. Most samples can be tested in less than 20 minutes while the minimum of 10 minutes must be used for the most

TABLE 3—RESULTS OF TESTS ON TRAY DRYER METHOD

Sample No.	Initial Weight of Sample	Final Weight of Sample	% Moisture		Time for Test
			Tray Dryer	Oven Dryer	
	grams	grams			minutes
1 Hay	100	25.5	74.5	75.0	20
2 Hay	100	34.0	66.0	65.0	17.5
3 Hay	100	48.5	51.5	49.6	13
4 Hay	100	59.0	41.0	38.8	15
5 Hay	100	66.0	34.0	34.8	13
6 Hay	100	73.0	27.0	27.2	11
7 Hay	100	77.5	22.5	20.8	19
8 Hay	50	42.0	16.0	15.2	12
9 Grain	100	83.0	17.0	16.6	17
10 Grain	100	83.5	16.5	15.0	18
11 Grain	100	84.5	15.5	13.4	18
12 Grain	100	88.0	12.0	12.1	15

rapid samples. To obtain an accurate determination quickly, the temperature should be the maximum possible without charring the sample. The temperature should not exceed 145° C. for hay or 160° C. for grain and the material must be evenly distributed in the tray. The accuracy was not affected by the moisture content of the material. Although a slight amount of fine material sifted through the screen or wire mesh, it had no apparent influence on the accuracy of the method. This method is simple and reasonably accurate.

Experiment IV (A)—Tray Dryer Using Kitchen Stove as the Heating Unit

Equipment

A wire sample basket, accurate scales, reading in grams, a kitchen stove and a thermometer are required for the test. In order to direct the heat to the sample a 10-inch length of pipe, large enough in diameter to surround the basket, is placed on top of the kitchen stove or hot plate. To allow air circulation when the sample basket is placed on the top of the pipe at least 50 per cent of the lower 4 inches of the pipe must be cut away.

Procedure

The determination is made in the same manner as described in the tray dryer method. The 100-gram sample is dried over the stove and the loss in weight indicates the moisture content.

TABLE 4—RESULTS OF DRYING SAMPLES ON A KITCHEN STOVE

Weight of Sample	Final Weight	% Moisture		Type of Stove
		Stove Method	Lab. Oven	
grams	grams			
98	76.5	21.9	24.3	Electric 1000W.
95	75.0	26.3	24.2	Electric 1000W.
50	41.0	18.0	16.1	Electric 1100W.

Discussion

It was found during these tests that a hot stove could be used to bring the sample to the drying temperature but the final heat should be reduced in order to complete the determination without burning the material. With this equipment the length of time required to dry the sample varied from one-half to three-quarters of an hour. This method has the advantage that no special stove is required to produce the heat for the test. Although an electric stove was used, the heat necessary could be developed by the average cook stove.

Experiment IV (B)—Tray Dryer Method Using a Kitchen Oven

This method uses the same equipment and procedure as Experiment IV and IV(A) except that the sample is dried in a kitchen stove oven. With the oven temperature at 250°–300° F. the moisture is driven off in one-half to three-quarters of an hour. To ensure uniform drying, bulky materials such as dry hay should be chopped and spread evenly in the drying tray. While this method is not very rapid, a determination can be made with only the purchase of an accurate scale.

TABLE 5—RESULTS OF DRYING SAMPLES IN A KITCHEN OVEN

Initial Weight	Final Weight	% Moisture		Time required to dry samples
		Stove Oven	Lab. Oven	
grams	grams			
95	65.6	31.0	30.7	30 minutes
98	66.0	32.7	33.8	35 minutes

Experiment V—Oil Immersion Method (4)

Equipment

Elaborate equipment is not necessary for this method. Accurate scales, reading in grams, a 200° C. thermometer, a metal container of one-quart capacity with a concave lid and a screen the diameter of the container are all the apparatus necessary. A supply of vegetable oil to cover the sample must be available.

Procedure

100 grams of chopped forage or grain are placed in the container and covered with vegetable oil. The screen is dropped on top of the sample and pressed below the oil with the thermometer. Almost any type of vigorous heat is sufficient to bring the temperature to 145° C. for hay or 180° C. for grain. This usually takes from 15 to 20 minutes. Then the sample is weighed and the moisture content calculated.

Discussion

Table 6 shows that the readings have an error of approximately $\pm$ 3 per cent. This error is allowable for the high moisture content in hay for silage but is rather wide for hay with a lower moisture content. With grain the error is reduced and it provides a suitable estimation of the condition of the crop.

TABLE 6—RESULTS OF THE OIL IMMERSION METHOD

Sample No.	Initial Weight of Sample	Final Weight of Sample	% Moisture		Time for test
			Oil Method	Oven Method	
	grams	grams			minutes
1 Hay	50	18.5	63	65.0	20
2 Hay	50	29.5	41	43.4	20
3 Hay	50	32.0	36	33.5	20
4 Hay	50	38.5	23	25.4	21
5 Hay	25	22.0	12	15.2	10
6 Oats	100	78.2	21.8	20.3	20
7 Oats	100	80.8	19.2	17.2	20
8 Oats	100	84.7	15.3	14.9	20
9 Oats	100	85.7	14.3	13.4	18
10 Oats	100	86.0	14.0	12.1	18

The method is best adapted to coarse stemmed materials, crops with high moisture content and grains. Dry or bulky materials require that the weight of the sample be reduced, and this results in an increased error due to the small sample.

When conducting a test, care must be exercised to control the oil temperature as it approaches the maximum allowed, since the oil temperature rises rapidly when the moisture in the sample is reduced.

The method is rapid and reasonably accurate for grain and silage, but is not so accurate for dry hay or other bulky materials.

Experiment VI—Common Salt Method (5)

Equipment

Since the results of these tests are estimated visually, no expensive thermometers or scales are necessary. A supply of dry salt (Na Cl) and a tight container (wax carton) are sufficient for the determination.

Procedure

A representative sample of long hay is selected and the stems bent by twisting them. The ends are trimmed to allow the hay to fit loosely in the container. A spoonful of salt is added, the container shaken 100 times and the contents examined. If clusters of salt have formed, the moisture content of the sample is over 25 per cent. If the salt is not affected, the moisture content is less than 25 per cent.

When testing grain the sample does not require any preparation. A handful of grain and a spoonful of salt are placed in the container and shaken for half a minute. The clusters of salt form when the moisture content of the grain is above 16 per cent.

Discussion

When the humidity of air rises above 75 or 80 per cent sodium salt becomes sticky and moist. Dexter (5) states that, "If hay and grains are to keep in storage, the atmosphere surrounding them must not remain at a relative humidity as high as 80–85 per cent for any great length of time." By placing a sample of the material and a spoonful of salt in a closed container, the salt will indicate whether the sample will produce a humidity that may cause spoilage.

This test did not give an accurate estimation of the percentage moisture in the hay sample, but an experienced operator could determine whether material was suitable for storage. It was difficult to estimate the moisture content of borderline cases. However, the salt appeared moist and with practice the condition of the sample could be determined.

Oat samples were tested in 2 to 3 minutes. The data show the borderline cases to range from 16.1 to 14.9 per cent indicating that an accurate estimation can be made since oats may be safely stored at 15.5 per cent moisture.

When the sample is uniformly damp the tests are reliable but oats with surface moisture will result in a slightly high reading while unripe oats with a dry surface will indicate a slightly low reading.

TABLE 7—RESULTS OF TESTS ON SALT METHOD

Material Tested	Condition of Salt		
	Salt damp with clusters	Salt lucid, no clusters	No change in salt
	% Moisture	% Moisture	% Moisture
1. Clover	65.6		
2. Timothy and Alfalfa	65.0		
3. Clover	45.6		
4. Timothy and Alfalfa	30.9		
5. Timothy		26.0	
6. Alfalfa		25.4	
7. Alfalfa		24.9	
8. Timothy		24.8	
9. Clover			20.8
10. Alfalfa and Timothy			19.0
11. Clover			19.5
12. Timothy			16.0
13. Oats	18.5		
14. Oats	18.0		
15. Oats	16.6		
16. Oats		16.1	
17. Oats		15.3	
18. Oats		14.9	
19. Oats			13.4
20. Oats			12.7
21. Oats			11.2

Although the test is rapid (5–10 minutes for each test) the degree of accuracy obtainable with forage crops does not warrant its general use. With cereal grains the method provides a useful indication of the moisture content.

Experiment VII—Relative Humidity Method (6)

Equipment

Two thermometers mounted in a rubber cork and fitted on an insulated quart bottle are used. Also a supply of concentrated salt (sodium chloride) solution and several wicks are required to conduct the test.

Procedure

One-third of a bottle of grain is necessary for each determination. A wick saturated with the salt solution is attached to one thermometer while the other thermometer remains dry. With the stopper in place the bottle

is shaken for about one minute and the thermometer reading noted at intervals. When the temperature is constant, it is recorded. This requires less than two minutes.

If the thermometers have identical readings the moisture content is approximately 16 per cent. A lower reading on the wet bulb indicates that the grain is suitable for storage. A higher reading shows that the grain is damp and cannot be safely stored.

TABLE 8—RESULTS OF TESTS ON THE RELATIVE HUMIDITY METHOD

Crop	Wet Bulb Tw	Dry Bulb Td	Difference Tw − Td	Actual Moisture Content
	Degrees C.	Degrees C.		
Oats	23.6	24.9	−1.3	11.2%
Oats	23.1	24.3	−1.2	12.7
Oats	25.9	25.0	−0.9	15.3
Oats	20.3	20.3	±0.0	16.1
Oats	21.4	23.0	+1.6	18.0
Oats	28.0	26.2	+1.8	18.5
Oats	23.9	22.1	+1.8	23.3

Discussion

For farm grains a saturated solution of sodium chloride is suitable for the wet bulb. However, Dexter (6) has conducted tests which prove that other solutions, such as magnesium chloride, potassium chromate, and barium chloride may be used to indicate other ranges of moisture content.

From the data it can be seen that the point at which the thermometers registered the same temperature is between 15 and 16 per cent moisture content. This indicates that the method can be used to estimate the keeping qualities of grain.

It was discovered during the tests that a thin wick produced much more rapid readings than the conventional woven wick. A couple of layers of cheesecloth are satisfactory. It was noted that surface moisture on the grain or sample resulted in a higher indication of moisture than was actually the case.

Although this method will not provide the actual moisture content of the grain, it does furnish a definite indication as to whether the crop will keep in storage.

SUMMARY

Seven methods of moisture determination of silage, hay and grain have been investigated at the Central Experimental Farm, Ottawa. They may be divided into four groups as follows:

(1) Calcium Carbide Method
(2) Pressure Method
(3) Rapid Drying Methods—3
(4) Humidity Methods —2

The calcium carbide method is precise within ± 3.0 per cent between 20 per cent and 75 per cent moisture with a hay crop. It is rapid and convenient to use.

The pressure method provides little indication of moisture content and is limited to a narrow range of moisture.

The three rapid drying methods supply a relatively precise reading of the moisture content over a wide range of moisture. They are inexpensive and simple to operate.

The humidity methods provide an indication of the storage condition of the crop. They may be used successfully with grain crops but are not so precise with hay.

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COMPARISON OF ORGANIC MATTER FRACTIONS FROM THREE SOIL TYPES[1]

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In a previous paper (8) a comparison was made of two organic matter fractions isolated by mild reagents from a soil of the black soil zone of Western Canada. It was concluded that there were no distinct chemical differences between the two fractions. The present investigation was undertaken to compare the organic matter of three distinctive types of soil after it had been subjected to a somewhat more extensive fractionation. It has been suggested (2, 9) that the humate fraction of the "electronegative" colloids of soil is important from the point of view of soil fertility. It seemed that a comparison of the chemical nature of the organic fractions isolated from different soils might offer some explanation of this observation.

Three soils were chosen, a podsol (A_1 horizon of a Greensboro loam from Lennoxville, Quebec) a dark brown prairie soil (0–6″ virgin surface soil from Scott, Saskatchewan) and a black soil (0–6″ surface soil from Lacombe, Alberta, taken from an area that had received no manure and no fertilizer). One hundred pounds of each soil were secured, screened to remove grass and roots, and quartered to secure laboratory samples weighing somewhat more than 3 kg. which were passed through a 2 mm. screen.

METHODS

The method used to separate the humate fractions (HF) was similar to that described by Atkinson and Turner (1) except that three kilogram samples were used. The KCl flocs were dried at 100° C., weighed and ground. The humate fractions were dried at 60° C. in a vacuum oven, weighed and ground to pass a 60-mesh screen.

The chemical methods used were similar to those described in a previous paper (8) except for the carbon determinations on the water and 1 per cent HCl extracts. For the analysis of these materials, an aliquot was evaporated to dryness and the carbon determined on the dry material by a modification of the wet combustion procedure described by McCready and Hassid (5).

RESULTS AND DISCUSSION

Amount of Isolated Fractions

The amount of material recovered in each fraction is reported in Table 1. The loss on ignition and per cent moisture are reported in Table 2.

Table 1 shows that the distribution of material between the various fractions of the different soils was similar to that reported previously (1). The amount of group 1 colloids, both organic and inorganic, is greatest in the Lacombe soil, somewhat lower in the Scott soil, and very low in the Lennoxville soil. The KCl flocs of the 2a and 2b colloids are highest in the Scott soil and lowest in the Lennoxville; however, the organic fractions of these groups are largest in the Lennoxville soil. The data show that the

[1] Scientific contribution No. 207, Division of Chemistry, Science Service.

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TABLE 1.—AMOUNTS OF MATERIAL IN FRACTIONS SEPARATED FROM THE SOIL
(as gm./100 gm. soil)

—	Scott	Lacombe	Lennoxville
Orig. Soil	100.0	100.0	100.0
Residue	70.8	64.3	73.0
KCl floc gp 1	5.7	12.2	0.7
KCl floc gp 2a	5.5	4.6	1.4
KCl floc gp 2b	1.5	1.0	0.2
1 HF,* gp 1	2.6	2.9	0.3
2 HF, gp 1	0.4	1.4	0.2
1 HF, gp 2a	2.3	3.5	6.9
2 HF, gp 2a	0.3	0.2	0.2
1 HF, gp 2b	0.3	0.3	1.5
Filtrates† from			
1 HF, gp 1	7.2	11.1	6.6
2 HF, gp 1	2.2	3.5	0.5
1 HF, gp 2a	9.9	13.1	26.8
2 HF, gp 2a	1.3	1.4	0.5
1 HF, gp 2b	5.3	7.7	8.1

* HF=Humate fraction
† Expressed as litres per 100 gm. soil.

TABLE 2.—MOISTURE AND LOSS ON IGNITION OF SOIL FRACTIONS
(per cent)

—	Scott		Lacombe		Lennoxville	
	Moisture	Loss on ignition	Moisture	Loss on ignition	Moisture	Loss on ignition
Orig. soil	1.1	11.3	1.6	13.4	1.5	15.4
Residue	0.5	6.6	0.5	5.0	1.6	7.0
KCl floc gp 1	1.9	8.9	2.0	9.5	1.3	10.2
KCl floc gp 2a	2.1	13.6	2.4	15.9	1.3	32.3
KCl floc gp 2b	2.0	16.8	1.4	14.9	1.3	26.2
1 HF, gp 1	8.8	38.5	12.8	48.9	6.8	75.2
2 HF, gp 1	5.8	63.2	7.7	72.6	4.6	56.9
1 HF, gp 2a	4.5	36.8	7.7	50.3	6.2	61.4
2 HF, gp 2a	8.9	41.7	11.0	62.3	4.9	63.1
1 HF, gp 2b	7.5	56.7	9.0	62.1	4.8	52.3

amount of inorganic colloid is very low in the Lennoxville soil although it contains more organic colloid than either of the other soils. About 90 per cent of the weight of the Lacombe and Scott soils, and 84 per cent of that of the Lennoxville soil could be accounted for by the combined weights of the colloids and the residue. The volumes of filtrate indicate that a shorter extraction time was required to remove all of the group 1 colloids from the Lennoxville soil than from the Scott and Lacombe soils, while the group 2a colloids of the Lennoxville soil required a much more extended extraction.

Table 2 shows that the separation of organic and mineral material of the colloid was poor; in some instances the loss on ignition of the "organic" colloid was less than 40 per cent. The organic fractions of the Scott soil were most contaminated and those of the Lennoxville soil somewhat the least.

The nitrogen and carbon content of the soil fractions

The nitrogen and carbon contents of the various fractions are recorded in Tables 3 and 4, the organic fractions being reported on an ash-free, moisture-free basis and the others on a dry weight basis. About $\frac{1}{4}$ of the

TABLE 3—NITROGEN CONTENT OF SOIL FRACTIONS
(Per cent and mgm./100 gm. of original soil)

	Scott		Lacombe		Lennoxville	
	%	Mgm.	%	Mgm.	%	Mgm.
Original soil	0.45	450.0	0.50	500.0	0.48	480.0
Residue	0.25	173.0	0.24	156.0	0.23	168.0
KCl floc gp 1	0.43	24.4	0.45	55.3	0.94	6.6
KCl floc gp 2a	0.64	35.1	0.78	35.6	1.17	16.5
KCl floc gp 2b	0.85	12.7	0.78	7.8	0.75	1.5
1 HF, gp 1	3.77*	37.7	3.60*	51.0	3.85*	8.7
2 HF, gp 1	4.70*	11.9	4.39*	44.7	6.23*	7.1
1 HF, gp 2a	4.70*	39.7	4.49*	79.1	3.93*	166.3
2 HF, gp 2a	4.99*	6.3	4.97*	6.2	3.18*	5.4
1 HF, gp 2b	5.11*	8.7	4.85*	9.0	3.34*	31.2
Filtrates from						
1 HF, gp 1		14.8		25.5		32.4
2 HF, gp 1		10.4		22.6		4.6
1 HF, gp 2a		4.1		36.1		84.1
2 HF, gp 2a		5.9		5.7		3.2
1 HF, gp 2b		15.0		14.1		21.2

* On ash-free, moisture-free basis.

TABLE 4.—CARBON CONTENT OF SOIL FRACTIONS
(Per cent and mgm./100 gm. of original soil)

	Scott			Lacombe			Lennoxville		
	%	Mgm.	C/N	%	Mgm.	C/N	%	Mgm.	C/N
Original soil	4.8	4800	10.6	6.1	6100	12.2	6.4	6400	13.3
Residue	3.4	2435	13.6	2.4	1550	10.0	4.7	3329	20.4
KCl floc gp 1	3.2	181	7.4	3.4	417	7.5	8.9	57	9.5
KCl floc gp 2a	4.9	270	7.7	7.0	323	9.0	16.3	228	13.9
KCl floc gp 2b	7.3	110	8.6	6.9	69	8.8	10.3	9	13.8
1 HF, gp 1	46.0*	460	12.2	54.0*	766	15.0	48.8*	110	12.7
2 HF, gp 1	53.9*	136	11.5	56.3*	571	12.8	52.2*	59	8.4
1 HF, gp 2a	49.4*	418	10.5	55.3*	973	12.3	53.3*	2256	13.6
2 HF, gp 2a	51.1*	64	10.2	55.2*	69	11.1	52.0*	66	16.3
1 HF, gp 2b	49.4*	84	9.7	56.0*	104	11.6	52.7*	474	15.8

* On ash-free, moisture-free basis.

nitrogen of the Scott soil and between $\frac{1}{3}$ and $\frac{1}{2}$ of the nitrogen of the other two soils was recovered in the humate fractions; in the Lacombe and Scott soils the nitrogen content of the humate fractions was fairly uniform but was lowest in the first humate fraction of group 1. The nitrogen contents of the Lennoxville humate fractions varied more widely and were, in general, lower than those of the other two soils. The carbon content of the humates of the Lacombe soil was somewhat higher than that of the humates isolated from the other two soils and its C/N ratio was somewhat higher than that of the Scott soil. In the Lacombe soil residue the C/N ratio was lower than that of the original soil, while in the other two soils it was higher. The C/N ratios of the inorganic colloids were lower than those of the original soils and residues in all but two instances while in general their nitrogen and carbon contents were quite high. Since these materials have been subjected to lengthy extraction procedures this may indicate a rather strong union between the inorganic and part of the organic colloids, especially the nitrogenous organic compounds. There is a tendency for the C/N ratio to decrease from the first humate fraction of group 1 to the group 2b humate fraction in two of the soils. This would indicate that the more carbonaceous organic colloids are removed first.

The uronic carbon and methoxyl contents of the soil fractions

The percentage of uronic carbon and the relationship of uronic carbon to total carbon in the different soil fractions are reported in Table 5. The higher proportion of uronic carbon to total carbon found in the KCl flocs of the Scott and Lacombe soils is difficult to explain since it might be expected that polyuronides would be removed by alkali; it would seem that this indicates a strong organic-inorganic union.

In general, the results for uronic carbon are similar to those recorded in the literature (4, 6). Forsyth (3) has determined the composition of a uronic acid-containing polysaccharide that he isolated from widely different types of soil. It contained galactose, glucose, mannose, arabinose, xylose

TABLE 5.—URONIC CARBON AND METHOXYL CONTENT OF THE SOIL FRACTIONS

	Scott			Lacombe			Lennoxville		
	Uronic Carbon %	Uronic C / Total C %	OCH_3 %	Uronic Carbon %	Uronic C / Total C %	OCH_3 %	Uronic Carbon %	Uronic C / Total C %	OCH_3 %
Original soil	0.58	12.1	—	0.66	10.8	—	0.84	13.1	—
Residue	0.27	7.9	—	0.26	10.8	—	0.33	7.0	—
KCl floc gp 1	0.59	18.4	—	0.51	15.0	—	1.02	11.5	—
KCl floc gp 2a	0.87	17.7	—	1.09	15.6	—	1.59	9.8	—
KCl floc gp 2b	1.21	16.6	—	1.16	16.8	—	1.21	11.8	—
1 HF, gp 1	4.90	10.6	0.73	4.90	9.1	0.69	6.20	12.8	2.15
2 HF, gp 1	5.40	10.0	0.89	4.40	7.8	0.68	4.60	8.8	1.25
1 HF, gp 2a	6.00	12.1	1.66	5.00	9.0	1.01	5.40	10.1	2.61
2 HF, gp 2a	6.70	13.1	1.03	4.60	8.4	0.98	5.90	11.3	1.98
1 HF, gp 2b	5.50	11.1	2.23	2.60	7.5	1.62	4.50	8.5	2.79

TABLE 6.—EXTRACTIVES IN HUMATE FRACTIONS

	Sol. in EtOH—C_6H_6			Sol in hot water				Sol. in hot 1% HCl		
	Total	Organic	Wt.	C	N	C/N	Wt.	C	N	C/N
	%	%	%	%	%	ratio	%	%	%	ratio
Scott—										
1 HF, gp 1	6.85	2.09	13.2	5.25	0.51	10.3	21.4	5.01	0.88	5.7
2 HF, gp 1	1.92	1.83	20.1	8.32	0.89	9.4	21.9	6.09	1.26	4.8
1 HF, gp 2a	2.39	2.07	14.6	7.31	0.86	8.5	25.9	7.82	1.32	5.9
2 HF, gp 2a	2.54	1.98	17.0	7.50	0.99	7.6	26.5	7.72	1.41	5.5
1 HF, gp 2b	4.36	3.75	20.1	8.51	0.92	9.3	23.9	7.02	1.25	5.6
Lacombe—										
1 HF, gp 1	6.04	2.81	11.2	5.46	0.48	11.4	18.7	4.11	0.70	5.9
2 HF, gp 1	1.65	1.55	17.5	6.67	0.73	9.1	15.3	3.81	0.77	4.9
1 HF, gp 2a	2.68	1.79	13.8	5.01	0.50	10.0	18.7	4.95	0.70	7.1
2 HF, gp 2a	—	—	—	—	—	—	—	—	—	—
1 HF, gp 2b	4.01	3.57	19.1	7.81	0.87	9.0	17.5	5.83	1.04	5.6
Lennoxville—										
1 HF, gp 1	11.75	10.26	22.3	7.28	0.74	9.8	17.1	5.81	1.08	5.4
2 HF, gp 1	—	—	—	—	—	—	—	—	—	—
1 HF, gp 2a	4.35	3.47	19.7	6.99	0.73	9.6	20.3	6.50	1.04	6.3
2 HF, gp 2a	—	—	—	—	—	—	—	—	—	—
1 HF, gp 2b	10.26	6.53	17.1	6.03	0.50	12.1	17.9	5.46	0.86	6.4

and glucuronic acid; the uronic carbon accounted for about 15 per cent of the total carbon. It seems unlikely that the major part of the uronic acid in soils is contained in compounds of this type, since, if that were true, a very large percentage, sometimes over 100 per cent of the total organic matter, would be thus accounted for. Polyuronides studied (7) vary greatly in their uronic acid content—from alginic and pectic acids which are composed of chains of uronic acids to arabic acid where the uronic carbon is less than 15 per cent of the total carbon. It would seem, from the low uronic carbon content of the soil polyuronide isolated by Forsyth and the relatively large amount of uronic acid carbon found in some soil fractions, that the soil uronic acids are combined in a variety of ways.

The methoxyl contents of the organic fractions are also given in Table 5. The results for the Lennoxville soil are somewhat higher than for the other two soils but, in general, they are low.

The content of extractives and of lignin in the humate fractions

The percentage of the organic fractions that is soluble in 1 : 2 ethanol-benzene, hot water and hot 1 per cent HCl is shown in Table 6 along with the amounts of carbon and nitrogen that were removed by the two latter reagents. Only small amounts of material were soluble in ethanol-benzene; the fractions from the podsol soil were much more soluble than the fractions from the other two soils. A relatively large amount of inorganic material was removed from the first humate fractions of the group 1 colloids of the Lacombe and the Scott soils. The percentage of nitrogen and carbon removed by the water and 1 per cent HCl extractions from the first humate fraction of group 1 of the Scott soil is lower than that dissolved from the other humate fractions. This does not seem to be true for the other two soils. In general it would seem that the organic fractions of the Lacombe

TABLE 7.—LIGNIN OF HUMATE FRACTIONS

——	Lignin*	Carbon†	Nitrogen†	Carbon / Nitrogen	Ash
	%	%	%	ratio	%
Scott—					
1 HF, gp 1	51.4	54.1	2.75	19.7	39.4
2 HF, gp 1	51.6	53.5	3.48	15.4	18.4
1 HF, gp 2a	41.6	60.6	3.40	17.8	56.2
2 HF, gp 2a	40.8	49.3	3.62	13.6	49.1
1 HF, gp 2b	42.7	50.9	3.81	13.4	32.5
Lacombe—					
1 HF, gp 1	63.4	57.6	2.71	21.2	23.8
2 HF, gp 1	59.7	55.7	3.34	16.7	13.7
1 HF, gp 2a	58.8	55.4	3.09	17.9	31.2
1 HF, gp 2b	52.5	53.4	3.85	13.9	27.0
Lennoxville—					
1 HF, gp 1	40.5	51.0	2.73	18.7	18.2
1 HF, gp 2a	47.1	53.4	2.68	19.9	27.6
1 HF, gp 2b	49.0	52.9	2.46	21.5	25.7

*Ash-free lignin, on basis of ash-free fraction.
†As per cent of ash-free lignin.

soil are somewhat less soluble in water and 1 per cent HCl than comparable fractions of the Scott soil with the Lennoxville soil fractions being intermediate in solubility. The carbon-nitrogen ratios of the water-soluble material are somewhat lower but do not differ greatly from those of the original fractions but in the 1 per cent HCl extracted material the ratios are considerably lower, indicating a selective removal of nitrogen by this solvent.

The percentage lignin in the humate fractions is reported in Table 7 along with the carbon and nitrogen contents and the C/N ratios of the ash-free lignin. The lignin content of the Lacombe soil fractions is somewhat higher than that of the Scott soil or the Lennoxville. There seems to be no relationship between the methoxyl (Table 5) and lignin contents of the humate fractions, except possibly in those of the Lennoxville soil. In the other two soils the group 1 organic fractions are highest in lignin and lowest in methoxyl content. In general, it would seem that soil lignin is demethoxylated to such an extent that the methoxyl content of soil organic matter has very little relation to its lignin content. The carbon content of the lignin fractions is quite similar to that of the humate from which they were derived, but their nitrogen content is lower. This results in a high C/N ratio in the lignin fractions.

SUMMARY AND CONCLUSIONS

There were many indications of a fairly strong union between some of the inorganic and organic colloids. Many of the organic fractions contained a considerable amount of "ash" which was not greatly reduced by the extractions with water, 1 per cent HCl and concentrated H_2SO_4 used to isolate the lignin. In spite of repeated extractions, the KCl flocs were high in their content of carbon and nitrogen; even the polyuronide complexes were not removed.

There were some differences in the organic matter of the three soils. The Lacombe soil fractions were higher in lignin and carbon and lower in methoxyl and uronic carbon than those of the other two; they were also lower in the amount of carbon, nitrogen, and total weight of material dissolved by water and 1 per cent HCl than the fractions of the other soils. The organic matter of the Lennoxville soil was highest in ethanol-benzene extractives. The organic fractions of the Scott soil were most contaminated with inorganic material, those of the Lacombe soil somewhat less so, and those of the Lennoxville soil were the least contaminated.

The data recorded seem to offer no explanation for the claim that the first humate fraction of group 1 colloids is more important from the standpoint of soil fertility than the other fractions. There are no distinctive differences between the fractions; such differences as do occur are the reverse of those to be expected on the supposition that these fractions may contribute to soil fertility to a greater extent than does the other material. They are higher in lignin (except in the Lennoxville soil where their amount is very small); they tend to be lower in material soluble in water and 1 per cent HCl; they are lower in nitrogen and carbon and they have higher C/N ratios than do the other fractions.

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ANIMAL PROTEIN FACTOR IN SWINE RATIONS

I. EFFECT ON GESTATION AND LACTATION
II. EFFECT ON PROGENY AFTER WEANING

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In 1943 Mitchell (20) concluded in a review that the general superiority of animal protein concentrates in promoting maximal production in swine and poultry may be due more to their higher content of certain minerals and vitamins than to their amino acid make-up. The general improvement in growth of swine that has been reported (5, 7, 8, 9, 12, 18, 19, 20, 31) when Animal Protein Factor (APF), or vitamin B_{12}, was added to rations devoid of animal-source protein, and the responses to trace minerals observed by others (13, 17) lend support to this thesis. These findings, together with Morrison's observation (23) regarding a decline in the quality of certain packinghouse products because of lower content of edible viscera, and the work of Peeler *et al.* (27) reporting vitamin B_{12} in wheat, oats, and alfalfa meal but none in yellow corn, have created a need for re-examination of supplements for swine in Canada.

McElroy and Draper (21) demonstrated some of the consequences of inadequate nutrition of the brood sow and several reports would seem to indicate that vitamin B_{12} may be involved in gestation-lactation performance (14, 18, 20, 28). It has been shown by using purified diets that vitamin B_{12} is a dietary requirement of the baby pig (1, 17, 19, 24, 25, 26).

This report concerns the need for APF by brood sows and their progeny under practical Western Canadian conditions.

EXPERIMENTAL

In October 1949, twenty-nine Yorkshire sows were divided into 4 groups of approximately equal average age. They were quartered in paddocks provided with straw shelters and were fed and watered twice daily outdoors at a distance from the shelters.

Prior to the allotment the sows had been on oat pasture and had received buttermilk and tankage in addition to their grain and mineral allowances. The rations employed in this experiment (Table 1) contained 15 per cent crude protein supplied by either linseed oilmeal or meat and bone scraps. One group of sows receiving each kind of protein supplement had APF* added to supply the equivalent of ten micrograms of vitamin B_{12} per pound of ration. In addition, riboflavin was included to meet Carrol and Krider's (3) estimate of the requirement by the brood sow. Morrison's (23) tables of vitamin analyses were used to estimate the amount of riboflavin required.

Owing to the possibility that iodized salt might fail to supply the iodine needs of the sow (10) the equivalent of one tablespoonful of a solution of one ounce of potassium iodide per gallon of water was given to each sow daily in the drinking water.

* Merck's APF No. 3.

TABLE 1—COMPOSITION OF RATIONS

Ingredients	Linseed oil meal		Meat and bone scraps	
	No APF	APF	No APF	APF
Barley, ground, lb.	900	900	1100	1100
Oats, ground, lb.	440	440	440	440
Alfalfa meal, lb.	200	200	200	200
Linseed oil meal, lb.	400	400	—	—
Meat and bone scraps, lb.	—	—	215	215
Iodized salt, lb.	15	15	15	15
Ground limestone, lb.	30	30	30	30
Bone meal, lb.	15	15	—	—
APF supplement, gm.	—	767	—	767
Riboflavin, mg.	200	200	300	300

No sows were bred until they had been on their respective experimental rations at least one month. Individual weights were taken initially, at the time of breeding, at monthly intervals until farrowing, and again at weaning eight weeks later.

Farrowing was done in farrowing pens in the main piggery. At birth the pigs were weighed individually and ear tagged with poultry wing bands, males first. Within 24 hours of birth each odd-numbered pig was given about 100 micrograms of vitamin B_{12} orally as a suspension of APF** in water. Reduced iron was administered in the usual manner when the pigs were 3, 10, and 17 days of age. Castration of males was done at 6 weeks of age. Additional weight records were made at 10 days and 56 days of age. The pigs were not creep fed but had access to the sow ration.

Following weaning the young pigs were retained on their dams' rations for a short period until allotments were made to growth trials. Admittedly the level of protein (15 per cent) was too low for optimal growth of weanlings but facilities did not permit changing the feed formulas. For the growth studies 32 weanlings representing each sow group were selected. These were 16 pigs that had received no APF supplement at birth and a similar group that had been treated. Two gilts and two barrows from each of these groups were allotted to each of the four growing rations. The formulas of the rations were similar to those employed for the sows.

Like the sows the growing pigs were housed in dry lot with access outdoors, group-fed, and weighed every 28 days. It was intended that the pigs be fed to 200–210 pounds liveweight but the onset of adverse weather necessitated the removal of some of the pigs from the experimental quarters. As a consequence the gains have been reported on the basis of the first 100 days' growth computed from growth curves plotted individually for the 128 pigs on test.

RESULTS AND DISCUSSION

Reproduction and Lactation

The responses of the sows to APF added to the breeding rations are presented in Table 2.

**Merck's APF Master Blend No. 19, 2.3 million LLD units of vitamin B_{12}/gram.

TABLE 2.—EFFECT OF APF AND KIND OF PROTEIN ON REPRODUCTION IN SOWS

Treatment	Description of sows			Initial-to-weaning weight change	No. of pigs farrowed (av./sow)	Av. birth weight of live pigs	% Still born
	No.	Av. age	Initial wt.				
		Yr.	lb.	lb.		lb.	
Linseed oilmeal (LOM)	16	2.9	433	+12	10.6	1.9	6
Meat and bone scraps (MS)	13	3.0	418	+19	12.5	2.0	12
No APF added to ration	12	3.3	459	+ 7	11.4	1.9	11
APF added	17	2.8	403	+22	11.5	2.0	7
LOM No APF	7	3.1	452	+ 3	10.0	1.9	7
APF	9	2.4	419	+19	11.0	1.9	5
MS No APF	5	3.4	469	+11	13.4	1.9	16
APF	8	2.8	386	+24	12.0	2.0	10

With respect to the kind of protein supplement used it will be noted that meat and bone scraps-fed sows produced larger litters and gave birth to slightly larger pigs but these advantages were offset by a higher percentage of stillborn pigs. In addition the sows fed meatscraps were in somewhat poorer flesh at the commencement of the test as indicated by the initial weights with regard to age. This factor could account for the differences noted in farrowing performance.

It likewise appears that the addition of APF resulted in no appreciable changes in the condition of the sow, considering initial weight for age, or in number of pigs farrowed. There was perhaps a trend toward lower percentages of stillborn pigs farrowed by sows fed APF.

The responses of the baby pigs during nursing is reported in Table 3. Evidently more progeny of sows fed linseed oilmeal survived and the advantage is not lost by basing the percentage survival on the number of pigs born *alive* rather than on the total number farrowed as was done in Table 3. APF in the sow's ration effected no apparent improvement in lactation or nursing performance.

The administration of a large dose of APF (100γ vitamin B_{12} equivalent) within 24 hours of birth appears, if anything, to have been detrimental to subsequent growth and survival. This conclusion seems justifiable regardless of the kind of protein or the presence of APF in the sow's ration.

The reproduction and lactation phases of this experiment have shown no striking improvement due to the addition of APF (primarily as a source of vitamin B_{12}) to the ration of the sow, despite the fact that all sows received their respective rations for more than four months prior to farrowing. Since Van Poucke *et al.* (30) obtained a favourable response in brood sows when APF was added to a corn-soybean oilmeal ration there could be more extensive synthesis occurring in the gastro-intestinal tract of swine fed typical Canadian feedstuffs. Attention has already been drawn to the possibility of vitamin B_{12} existing in certain feeds of plant origin (27). It is also of interest that reproduction in mice is not readily affected by the consumption of diets low in vitamin B_{12} (11, 15).

TABLE 3.—EFFECT OF APF AND KIND OF PROTEIN IN SOW RATIONS, AND OF APF TO PIGS AT BIRTH, ON 10-DAY AND WEANING WEIGHTS AND ON PERCENTAGE SURVIVAL OF PIGS FARROWED

Treatment	10 days of age		56 days of age	
	Av. weight	% survival	Av. weight	% survival
	lb.		lb.	
Linseed oil meal (LOM)	5.7	75	23.2	70
Meat and bone scraps (MS)	5.4	70	24.0	62
No APF added to sow ration	6.1	72	24.0	69
APF added	5.2	73	23.3	63
No APF at birth to pigs	5.6	78	24.3	71
APF at birth	5.6	68	22.8	61
No APF to sow No APF at birth	5.9	78	23.7	75
No APF to sow APF at birth	6.3	68	24.5	66
APF to sow No APF at birth	5.4	78	24.8	68
APF to sow APF at birth	5.1	68	21.4	57
LOM to sow No APF at birth	6.3	82	23.8	79
LOM to sow APF at birth	5.3	68	22.8	60
MS to sow No APF at birth	5.8	73	24.3	63
MS to sow APF at birth	5.2	68	23.8	62

Although the vitamin B_{12} content of non-ruminant milk is reported to be variable (6) the several levels of vitamin B_{12} consumed by the sow groups were not reflected in different average weaning weights. Thus this vitamin was not the limiting factor responsible for the failure of the majority of the weanlings to achieve more normal growth (2).

Growth

Variations in initial weights may be expected in this type of study since selection of animals was restricted chiefly to sex; only such animals as were distinctly unthrifty or unusually large were ineligible. The average initial weight of the 128 pigs allotted to the growth test was 24.3 pounds. Covariance analysis (4) of the 100-day gains and initial weights revealed a highly significant correlation ($r = 0.948$) but no significant differences in initial weights occurred between groups involving major comparisons. The plan of variance is shown in Table 4.

The effects of APF and protein treatments on sows and their progeny were assessed from the gains after adjustment for initial weight differences. The important features of the growth data are shown in Tables 5 and 6.

The general response of the progeny after weaning to APF received by their dams was favourable. The pigs from sows fed linseed oilmeal + APF outgained the progeny of linseed oilmeal-fed sows by 11 pounds and there was a similar but non-significant ($P = 0.05$) trend in the case of pigs from sows fed meat and bone scraps (Table 5). The inclusion of APF in the growing ration assisted but it did not wholly compensate for failure of the sows on linseed oilmeal rations to receive APF during gestation and

TABLE 4—VARIANCE PLAN FOR PIGS ON GROWTH STUDY

Source of variance	Degrees of freedom
Total	127
Sow rations	3
Protein	1
APF	1
Protein × APF	1
APF at birth	1
Growing rations	3
Protein	1
APF	1
Protein × APF	1
Sex	1
Sow rations × growing rations	9
Missing values	2
Error (including remaining interactions)	108

lactation. It had a deleterious effect when added to growing rations containing meat and bone scraps (Table 6). Providing APF to baby pigs shortly after birth was of no benefit regardless of the supplementation of the sow or of the pigs. Most rapid growth was made by the progeny of sows fed meat and bone scraps supplemented with APF which were subsequently allotted to growing rations supplemented with linseed oilmeal.

In summarizing, these data indicate that APF additions to sow rations, especially to those containing linseed oilmeal as the protein, were more beneficial to the growth response of the progeny following weaning than was administration of APF at birth or inclusion of APF in the growing rations.

Further evidence is provided in Tables 5 and 6 that meat and bone scraps were inferior to linseed oilmeal as a supplement to the growing ration. Pigs fed meat and bone scraps grew no better than those fed linseed oilmeal and in fact were decidedly slower in growing if their dams had also been fed meat and bone scraps. From the standpoint of amino acid content it is generally conceded that proteins of animal origin excel those of plant origin in biological value but from the work of Williams (34) and of Wilder *et al.* (33) it appears that meat and bone scraps may be lower than linseed oilmeal in tryptophane content. The digestibility of the protein in meat and bone scraps fed to swine is lower than that in linseed oilmeal (29, 32); thus it is possible that amino acid inadequacy and a lower digestible protein level in the meat and bone scraps rations may have contributed to the poor gains obtained.

TABLE 5.—EFFECT OF KIND OF PROTEIN AND OF APF SUPPLEMENT IN SOW RATIONS ON GROWTH OF PROGENY. GAINS ADJUSTED FOR INITIAL WEIGHTS

Treatments of progeny	Supplements in sow rations				Necessary difference P = .05	No. pigs/ group
	Linseed oilmeal		Meat and bone scraps			
	No APF	APF	No APF	APF		
Average 100-day gain (lb.)	80	91	85	88	3.2	32
No APF at birth	82	91	85	91	4.6	16
APF at birth	78	90	85	84		
No APF in growing ration	78	97	86	90	4.6	16
APF in growing ration	82	84	84	85		
Linseed oilmeal	80	88	95	104	4.6	16
Meat and bone scraps	80	93	75	71		

TABLE 6—EFFECT OF PREVIOUS APF AND PROTEIN TREATMENTS ON PIGS FED PLANT OR ANIMAL SOURCE PROTEINS WITH OR WITHOUT ADDED APF. GAINS ADJUSTED FOR INITIAL WEIGHTS

Treatment prior to growth test	Supplements in growing rations				Necessary difference P = .05	No. pigs/ group
	Linseed oilmeal		Meat and bone scraps			
	No APF	APF	No APF	APF		
Average 100-day gain (lb.)	92	91	83	76	3.2	32
No APF at birth	94	94	84	78	4.6	16
APF at birth	91	89	83	74		
No APF added to sow's ration	85	91	80	75	4.6	16
APF added to sow's ration	100	92	86	77		
Linseed oilmeal in sow's ration	86	83	90	83	4.6	16
Meat and bone scraps in sow's ration	99	100	77	70		
L.O.M. (sows) with no APF	82	78	75	86	6.5	8
L.O.M. (sows) with APF	89	88	105	80		
Meat scraps (sows) with no APF	88	103	85	65		
Meat Scraps (sows) with APF	111	97	69	74		

SUMMARY AND CONCLUSIONS

An experiment has been reported in which the value of the animal Protein Factor (APF), primarily as a source of vitamin B_{12}, as a supplement to swine rations was studied. Four rations employing barley and oats as the basal grains were supplemented with linseed oilmeal or meat and bone scraps and fed with or without APF. Twenty-nine Yorkshire sows were

allotted to these rations prior to breeding and their progeny were tested on similar rations following weaning. One-half the pigs from each sow received about 100 micrograms of vitamin B_{12} (as APF) orally shortly after birth. The following conclusions appear justified:

1. When included to supply 15 per cent total protein, linseed oilmeal and meat and bone scraps were of equal value to the brood sow. However the latter was inferior to linseed oilmeal in the growing ration. Possible reasons are discussed.

2. The addition of APF to the brood sow's ration did not affect her condition or the number of pigs farrowed. Small increases in percentages of pigs alive at birth were noted in APF-fed sows.

3. Administration of APF shortly after birth was not beneficial to baby pigs regardless of the supplementation of the dams' ration.

4. The inclusion of APF in the growing rations was ineffective in stimulating growth and appears to have been deleterious when added to rations containing meat and bone scraps.

5. The progeny of sows which received APF in their rations during gestation and lactation gained faster after weaning than others whose dams had not received additional APF.

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RESIDUAL VALUES OF CERTAIN INSECTICIDES AGAINST ADULTS AND LARVAE OF *PHYLLOPHAGA* SPP.

(A progress report)[1]

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INTRODUCTION

Phyllophaga fusca (Froel.) was the predominant species of June beetle in the Marmora area of Hastings County, Ontario, during 1950. *P. anxia* (Lec.) was also present but comprised less than 10 per cent of the June beetles collected from agricultural lands. Development of each of these species in this area is remarkably uniform (1); adults, eggs, and first- and second-instar grubs occur during the first year of the life-cycle; second- and third-instar grubs during the second year; and third-instar grubs, pupae, and inactive adults during the third year. Those areas with a major flight of June beetles in 1950 were Brood A areas, which comprise a large part of the agricultural areas of Ontario. Beetles were in the surface soil of meadow and pasture lands during early May; during late May and throughout June they fed on the foliage of red oak, white ash, butternut, and other deciduous species throughout the night, returning to the soil to hide throughout the day. Previous experiments indicated that ground, rather than foliage, treatments were more practical from the standpoint of beetle control, and all experimental work was planned on the basis of ground treatments of areas suitable for June beetle habitation and oviposition.

In preliminary experiments with BHC and DDT before 1947, it was found that either insecticide would readily kill the beetles before they could feed to any extent, mate, or deposit eggs. Extensive experiments in 1947 (2) showed that these insecticides were promising, beetle mortality being in proportion to the dosage employed. During that year only one collection of dead beetles was made, for the rather lengthy period of effectiveness of the insecticides was not fully known. In the plots in which dead beetles were collected in 1947, white-grub counts were made during the same and the following year. There was evidence that a significant portion of the insecticide worked downward in the soil to the feeding level of first- and second-year grubs.

Further testing of insecticides was carried out in 1949 at Pontypool in Durham County just before the major beetle flight. Beetle and grub collections indicated that BHC was the most effective insecticide tested, chlordane being somewhat less effective. Toxaphene and parathion were effective only at high rates of treatment under favourable field conditions.

A more extensive test was made at Marmora in 1950 on replicated plots, in separate units and in a series of 13 latin squares of 36 plots each. These were situated on old pasture sod known to have a widely varying population of *Phyllophaga* spp. within a reasonable distance of food trees

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of the adults. Two-gallon compressed-air sprayers were used throughout for liquid application; dusts were broadcast by hand, sand being used as a diluent to ensure more even application at the lower rates. In the 36-plot blocks the plots were randomized, and the one latin square was used throughout; there were six replications of each treatment, i.e., six checks and 30 treated plots, treatment being made at five strengths of insecticide. Both treatments against adults and those against adults and larvae were applied before significant flight of the adults.

TREATMENTS AGAINST ADULTS

BHC, chlordane, aldrin, and dieldrin were compared on an area with an uniform initial population of about 75,000 adults per acre, as determined by soil sampling of larvae and pre-pupae the fall before, at the rate of 4 pounds of actual insecticide per acre. Treatments were made on duplicate plots 20 × 20 feet on the crest of a ridge that was sparsely covered with food trees of the adults, such as white elm, white ash, butternut, and large-toothed poplar. Nightly observations on general beetle activity were made in the vicinity during the flight period. Insecticides were applied as sprays on May 25. Collecting of dead beetles was commenced two days later and continued at intervals of several days until June 24. No untreated plots were used as checks; the object of the experiment was to compare the effectiveness of new insecticides with that of lindane, which had previously been shown (3) to have high residual value.

Table 1 shows the numbers of dead beetles collected on the nine collecting dates, the totals from the plots being given. The numbers of June beetles collected per square foot from the plots treated with each insecticide were as follows: dieldrin, 0.54; aldrin, 1.36; chlordane, 0.85; BHC, 4.09. No sampling was attempted in these plots during the latter part of the summer to determine first-year grub populations.

TABLE 1.—TOTAL NUMBERS OF DEAD JUNE BEETLES COLLECTED FROM DUPLICATE PLOTS TREATED ON MAY 25 WITH FOUR POUNDS OF ACTUAL INSECTICIDE PER ACRE, MARMORA, ONT., 1950

Material	May			June						Total
	27	29	31	2	7	9	13	17	24	
Dieldrin*	13	33	60	67	43	63	43	50	65	437
Aldrin*	62	63	99	197	143	156	95	133	142	1,090
Chlordane**	30	39	135	95	75	81	81	71	76	683
Lindane*	362	348	642	582	432	399	396	400	422	3,983
Total	467	483	936	941	693	699	615	654	705	6,193

* 25 per cent wettable powder.
** 40 per cent wettable powder.

TABLE 2.—TREATMENTS AGAINST ADULTS AND LARVAE OF *Phyllophaga* SPP., WITH DATES AND INITIAL POPULATION LEVELS, MARMORA, ONTARIO, 1950

Block	Treatment	Date	Initial population level for adults (per acre)
East Field		May	About 75,000
1	DDT, 50% wettable powder	15	" "
2	BHC dust, 3% gamma isomer	15	" "
3	Lindane, 25% wettable powder	16	" "
			About 50,000
4	Chlordane, 40% emulsifiable concentrate	17	" "
5	Aldrin, 25% wettable powder	17	" "
6	Dieldrin, 25% wettable powder	18	" "
			About 10,000
7	Dieldrin, 25% emulsifiable concentrate	18	" "
8	Aldrin, 25% emulsifiable concentrate	18	" "
West Field			About 25,000
9	BHC, 3% dust with 300 lb. of fine sulphur added	22	" "
10	Chlordane, 5% dust with 300 lb. of sulphur added	22	" "
11	DDT, 50% wettable powder	23	" "
12	Aldrin, 25% wettable powder	23	" "
13	Dieldrin, 25% wettable powder	23	" "

TREATMENTS AGAINST ADULTS AND LARVAE

A series of 13 large blocks, each consisting of a latin square of 36 plots each 20 × 20 feet, was set up on a large farm at Marmora. Numbers 1 to 8 were in one field on a steep slope where the population range was wide (determined by sampling to be from 10,000 to 75,000 adults per acre). The remainder were in a field 500 yards to the west, sufficiently distant to exclude ordinary flight between fields. The second field had an irregular, moderate slope, and the infestation early in May averaged about 25,000 per acre. Table 2 shows the treatments, with dates and initial population levels, the rates ranging from 1.25 to 20.0 pounds of actual insecticide per acre.

In Table 3 are given the numbers of dead beetles collected on four dates, grouped and averaged for each insecticide at 5 different strengths. Collections were made from entire plots.

A soil sample (one-quarter of a square yard per plot) was taken at random from each plot in the 13 blocks. One count was made, in one block at a time, from mid-July to mid-September. The average number of live grubs per square foot for each of the treatments and the checks is shown in Table 4.

The average number of dead beetles per plot and that of live first-year grubs are grouped in Table 5. The beetle counts are based on entire-plot collections, and those of the grubs on the numbers alive in the soil per one-quarter square yard.

TABLE 3.—TOTAL NUMBERS OF DEAD JUNE BEETLES IN FOUR COLLECTIONS (MAY 21 TO JUNE 27) FROM 6 REPLICATED PLOTS TREATED ONCE (MAY 15 – 23) WITH INSECTICIDE, AND FROM CHECKS, MARMORA, ONT., 1950

Insecticide	Block	Pounds per acre					Checks	Total
		1.25	2.5	5.0	10.0	20.0		
DDT	1	276	478	528	435	762	133	2,612
	11	65	165	190	127	194	64	805
Total		341	643	718	562	956	197	3,417
Average		170	321	358	281	478	98	1,708
BHC	2	1,958	2,480	3,457	3,467	4,079	835	16,285
Lindane	3	1,293	1,106	1,679	3,344	2,766	494	10,682
BHC	9	291	380	401	389	386	161	2,008
Total		3,542	3,975	5,537	7,200	7,231	1,490	28,975
Average		1,180	1,327	1,845	2,400	2,410	496	9,858
Aldrin	5	284	181	231	305	381	237	1,629
	8	69	75	87	106	99	62	438
	12	100	91	82	88	85	101	547
Total		453	357	400	499	565	400	2,674
Average		151	119	133	166	188	133	891
Dieldrin	6	1,048	625	471	613	779	281	3,817
	7	197	220	225	221	277	113	1,253
	13	85	99	109	106	110	77	586
Total		1,330	944	805	940	1,166	471	5,656
Average		443	314	268	313	388	157	1,885
Chlordane	4	376	461	542	428	557	194	2,558
	10	94	79	81	120	147	54	575
Total		470	540	623	548	704	248	3,133
Average		235	270	311	274	352	124	1,566

DISCUSSION OF RESULTS

Table 1 indicates that during the first month there was no apparent reduction in the toxicity of dieldrin, aldrin, chlordane, or BHC; the last gave the highest mortality of June beetles in nine collections, an average of 4.09 beetles per square foot. More than one ground application of insecticide was not necessary or desirable.

As indicated in Table 3, BHC consistently killed the largest numbers of June beetles; its effectiveness was significant at the 1 per cent level. Aldrin and dieldrin each killed considerable numbers of beetles, but of these insecticides only 25 per cent emulsifiable dieldrin was significantly effective at the 5 per cent level. Chlordane was not significantly effective against beetles, and DDT was significantly effective only at the 5 per cent level. Each dust was improved with the addition of 300 pounds of fine sulphur, but tests of dieldrin and DDT should be repeated.

TABLE 4.—AVERAGE NUMBER OF LIVE FIRST-YEAR WHITE GRUBS PER SQUARE FOOT AFTER TREATMENT WITH VARIOUS INSECTICIDES AGAINST THE ADULTS, MARMORA, ONT., 1950*

Insecticide	Block	Pounds per Acre					Checks
		1.25	2.5	5.0	10.0	20.0	
DDT	1	10.8	6.2	5.3	5.7	3.4	11.4
	11	2.6	2.2	0.5	3.3	0.6	3.1
Total		13.4	8.4	5.8	9.0	4.0	14.5
Average		6.7	4.2	2.9	4.5	2.0	7.2
BHC	2	2.2	2.5	3.1	2.6	0.3	7.4
Lindane	3	6.0	5.1	3.3	4.4	0.2	12.1
BHC	9	1.9	0.6	0.0	0.0	0.2	2.5
Total		9.1	8.2	6.4	7.0	0.79	22.0
Average		3.0	2.7	2.1	2.3	0.26	7.3
Aldrin	5	9.9	11.0	13.4	12.2	8.0	7.5
	8	1.6	1.6	2.8	1.2	3.1	1.6
	12	2.6	1.1	2.2	3.2	0.6	5.8
Total		14.1	14.9	26.8	18.5	9.5	14.9
Average		4.7	4.9	8.9	6.1	3.1	4.9
Dieldrin	6	13.4	9.5	11.4	14.0	9.8	14.8
	7	3.5	2.7	1.8	1.3	2.0	4.9
	13	2.9	1.1	1.7	3.1	0.6	0.5
Total		19.8	13.3	14.9	18.4	12.4	20.2
Average		6.6	4.4	4.9	6.1	4.1	6.6
Chlordane	4	7.5	10.6	8.9	5.8	7.0	13.1
	10	0.29	1.7	0.0	0.29	0.07	0.29
Total		7.79	12.3	8.9	6.09	7.07	13.39
Average		3.89	6.1	4.4	3.04	3.38	6.69

* One count was made, for each block at a time, from mid-July to mid-September.

Not all the dead beetles found on any one plot can be definitely stated to have received the fatal dosage of poison from that plot; some died on plots other than those on which they received the fatal dosage, and others died outside the experimental area. However, since in the statistical analysis the treatments were compared with the check plots and there were 6 replicates of both treatments and checks, the significant values found indicate in large part the actual efficiencies of the insecticides.

Table 4 shows that virtual elimination of first-year grubs was accomplished with chlordane and with BHC at the 5- to 20-pound rates. Reductions in larval populations in proportion to rates of application occurred in all plots except those treated with dieldrin or aldrin. The lowest counts of live larvae and the highest counts of dead beetles were obtained with BHC at each strength.

TABLE 5.—AVERAGE NUMBERS OF DEAD JUNE BEETLES IN FOUR COLLECTIONS (MAY 21 TO JUNE 27) AND OF SURVIVING FIRST-YEAR GRUBS IN ONE COLLECTION (MID-JULY TO MID-SEPTEMBER) FROM 6 REPLICATED PLOTS TREATED ONCE IN MAY WITH INSECTICIDE, AND FROM CHECKS, MARMORA, ONTARIO, 1950

Insecticide	Stage of insect	Pounds per acre					Checks
		1.25	2.5	5.0	10.0	20.0	
DDT	Beetles	170	321	358	281	478	98
	Grubs	6.7	4.2	2.9	4.5	2.0	7.2
Gamma BHC	Beetles	1,180	1,327	1,845	2,400	2,410	496
	Grubs	3.0	2.7	2.1	2.3	0.26	7.3
Aldrin	Beetles	151	119	133	166	188	133
	Grubs	4.7	4.9	8.9	6.1	3.1	4.9
Dieldrin	Beetles	443	314	268	313	388	157
	Grubs	6.6	4.4	4.9	6.1	4.1	6.6
Chlordane	Beetles	94	79	81	120	147	54
	Grubs	3.89	6.1	4.4	3.04	3.38	6.69

The time involved from initial contact with the insecticide to the death of the beetle or white grub was not closely studied. A period of 36 to 48 hours was indicated as necessary for beetles in most cases. During the first night after the ground surface had been treated, beetles emerged from the soil without visible symptoms of poisoning, but before they could seek cover with the approach of daylight they were usually unable to hide under the surface soil or litter. In general collecting, about 25 per cent of the beetles were lying on the soil surface, moving either legs or wings but incapable of flying, crawling, or burrowing. Depressions or small pockets in the soil surface usually contained more dead beetles than a flat surface, indicating that beetles crawled along or fluttered for a short distance and finally dropped into a depression, from which they were unable to get out. In a number of cases large numbers of dead beetles were found around the bases of young aspen poplars growing naturally in treated plots, indicating that the beetles did not move far from the food plant before dying.

Movement of beetles from the soil to food trees and back to the soil for hiding during the day was studied in some detail. They commonly flew to upper slopes or small hilltops, preferably those with food foliage. The warmer soil in such places during early summer was evidently preferred. When feeding night after night on the same species of tree they do not fly far during a single night, but they are forced to change food trees when the latter become completely defoliated. Exceptionally large numbers of live beetles may be found on the upland sites of rolling country, especially under preferred foliage; and it is obvious that surface applications of insecticides applied to such areas are likely to come in contact with unusually large numbers of beetles. Surface applications of the insecticides studied may adhere to the lower foliage of grasses, but much lies on the soil surface or soil litter, gradually working into the soil to a depth of several inches or more. A surface application of BHC may work into the soil to the grub-feeding level in about one month, as determined from the toxic effect on second-year white grubs (3).

In these experiments the grub populations were reduced both by the killing of the adults and directly by the insecticides. Further reductions were certain to occur during the rest of the larval feeding period in 1950 and again in 1951 before the beginning of the heaviest feeding, about July 1.

CONCLUSIONS

1. BHC was by far the most effective of the insecticides tested against adults and larvae of *Phyllophaga* spp. An application of 20 pounds per acre of gamma BHC almost eliminated white grubs in the first year. Surface application to permanent turf at relatively low rates should be effective in protecting arboreal foliage and in preventing grub damage to plant roots.

2. DDT, chlordane, aldrin, or dieldrin used at relatively higher rates per acre, may be applied to areas where tainting of crops is a primary consideration.

3. Aldrin and dieldrin should be tested further to determine whether they have sufficient residual effect to kill grubs at feeding levels.

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GREY MOULD WILT OF RASPBERRY[1]

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During the summers of 1942 and 1943, a small experimental planting of raspberries at Truro, Nova Scotia, was found attacked by a disease that appeared to be weakening or killing many of the canes. Some of the young shoots were dead and bleached at the tips. On the bleached areas a fungus, belonging to the genus *Botrytis*, was fruiting abundantly. No further observations were made on this disease until 1947 when it was found in other plantings. A review of the literature on raspberry diseases revealed that Harris (1), in England, had described a "Botrytis rot" on the stems of the variety Lloyd George, which appears to be similar to that found in Nova Scotia. He states that the disease is able to cause severe damage to a raspberry plantation. The name of "Grey Mould Wilt" given to the disease by Wormald (2) is appropriate. The disease is apparently new to Canada.

Preliminary isolations made from material collected at Truro in the summer of 1947 yielded a number of organisms in addition to the species of *Botrytis* in question. Subsequently pure cultures of a *Botrytis* species of the *cinerea* type were obtained in late season isolations from blackened areas under the epidermis of larger canes as well as from bleached areas on the younger canes. Collections of diseased canes made in the winter months revealed the blackened areas on the canes to be thin sub-epidermal sclerotia. These yielded pure cultures of the fungus which fruited sparingly but produced numerous sclerotia on potato dextrose agar.

Early in the spring of 1948, young raspberry plants were brought to the greenhouse from a planting in which the disease had not been found. Preliminary inoculations were made by spraying 6 plants with a spore suspension obtained by rinsing a pure culture of the fungus with sterile, distilled water. A tan discolouration developed on one cane around the base of an attached moribund leaf. This discoloured area later extended along the cane for about 10 cm. and produced symptoms similar to those described below.

Another series of plants was inoculated by placing a small sclerotium, from available cultures, in an incision on the internode of actively growing canes, about 8 inches from the soil surface. Conidia were produced from the sclerotia within a weeek but no apparent infections were observed at the point of inoculation. No further experiments were conducted to determine whether the organism would invade the host through wounds.

Further inoculations were attempted in the spring of 1949 when a group of plants, which had been growing for some weeks in the greenhouse and had produced a strong growth of canes, was available for the purpose. As soon as the lower leaves showed signs of yellowing the plants were sprayed with a water suspension of spores from cultures of the organism and kept moist for 24 hours. Discoloured areas appeared around the nodes of moribund leaves within 3 weeks. Isolations from leaf petioles and discoloured areas on the canes yielded the *Botrytis* organism.

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FIGURE 1. Raspberry canes showing sclerotia.

FIGURE 2. Sclerotia bearing *Botrytis* conidia.

When first infected, the canes develop a light brown to tan discolouration around the stem at the base of the infected petiole. The discoloured areas continue to spread up and down the stem. When adjacent nodes show infections the affected areas gradually coalesce. During the latter part of the summer dark blotches from 1 to 2 mm. wide and from 2 to 15 mm. long appear in the discoloured areas of the stems and develop into sclerotia. These sclerotia do not produce conidia until the following spring.

On potato dextrose agar at room temperature, the mycelium of the pathogen covers a slant within 7 days and produces a small area of conidiophores on the upper wedge of the medium. The base and sides of the culture later produce abundant sclerotia, 2 to 15 mm. long and 2 to 5 mm. wide. The conidia vary in size from 7–18 × 5–11μ with an average of 9.8 × 6.0μ. They are mostly obovate to spherical, although some are slightly pyriform in shape. No cultures of the fungus isolated by Harris (2) were available for comparison.

The disease has been seen only in plantings under a sawdust mulch culture. Such a culture system encourages a dense growth of canes. Under such conditions, the lower leaves on new canes cease to function actively and begin to yellow much earlier in the season than those in a more open planting. The dense growth and yellowing leaves provide, during wet weather, favourable conditions for infection by the pathogen.

The most severely infected canes are killed before spring but those more lightly infected survive. The subepidermal sclerotia may be found the following spring, both *in situ* (Figure 1) and fallen to the ground. After a period of rainfall and high humidity, conidia may be found on the sclerotia adhering to the canes (Figure 2) as well as on those that have fallen to the ground. These conidia appear to constitute the only source of infection.

During the last 8 years the disease has been more severe in seasons when the rainfall has been abnormally high between June and September.

DISCUSSION

The finding of this disease in raspberry patches under sawdust mulch culture conditions in Nova Scotia creates a new problem. Sawdust mulching has been encouraged in recent years on lighter soils as it has given very good results in plant growth and crop yield. The appearance of grey mould wilt imposes a disease control problem which has not been thoroughly investigated under such cultural methods. The pruning out and burning of affected canes in the autumn appears to be essential to the elimination of the overwintering sclerotia.

In the spring the sclerotia are loosened from the epidermis and many drop to the ground. Those remaining on the canes may be found bearing conidia abundantly following favourable weather and hence constitute a source of infection to new canes. The sclerotia found on the sawdust also produce conidia freely. It is conceivable that the latter may also cause infections at a near ground level as plants have been observed in the field that suggest this possibility. No observations have been made on the longevity of the sclerotia beyond the first season.

The summer symptoms of grey mould wilt are readily distinguished from spur blight by the tan colour of the lesions. The later development of sub-epidermal sclerotia in the wilt infected canes is a distinguishing symptom of this disease, common to no other raspberry disease known in Canada.

In the inoculation experiments the organism gained entrance to the host through attached moribund leaves but not through wounds. Further study on methods of inoculation and incubation periods would add light to the pathology of the disease.

The strain of *B. cinerea* used in these experiments was obtained from a sclerotium. No other strains of the organism were tested.

SUMMARY

1. The grey mould wilt of raspberries in Nova Scotia has been found to be caused by a strain of the fungus, *Botrytis cinerea*.

2. The disease is most prevalent during wet seasons.

3. The sawdust mulch method of culture appears to favour the disease.

4. The pathogen overwinters as subepidermal sclerotia in the canes.

5. The eradication and destruction by burning of affected canes during the late summer and early autumn appears essential to the control of the disease.

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SELF-STERILITY STUDIES IN ALFALFA[1]

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Studies were made at Ottawa during 1945–50 to determine the possibilities of using highly sterile alfalfa plants for the production of hybrid strains along the lines proposed by Tysdal and Kiesselbach (4). These authors suggested that crosses between such plants might yield progenies exhibiting the typical hybrid vigour of first-generation plants. Due to high self-sterility the amount of selfed and sibbed seed in first and second generations would be at a minimum.

Before undertaking an extensive breeding program along these lines it was felt advisable to conduct a limited study with particular attention to the nature of self-sterility in alfalfa and how the factors controlling it react in the first two generations. The studies therefore followed these steps of determining: (1) frequency of highly self-sterile plants in a field of a standard variety; (2) the evaluation of self-sterility, cross-fertility and cytological behaviour of a representative group of selections; (3) the inheritance of self-sterility in the F_1 generation, and (4) a study of cross-fertility in related and unrelated plants in the F_1 generation.

SEARCH FOR SELF-STERILE PLANTS

Ladak was used as the source for the selected plants. From a mass nursery of 2,000 spaced plants, 270 were selected at the pre-bloom stage on the basis of vigour, leafiness and semi-upright to upright growth habit. To obtain a measurement of the self-sterility, 4 to 6 unopened racemes were bagged on each plant (using $\frac{1}{2}$ lb. "Kraft" paper-bags). At three-day intervals during the flowering period the bags were removed and the open flowers tripped by stroking the racemes. Upon harvesting the bags a note was taken on the open-fertilized portion of the plant as regards pod-setting the plants being graded as "poor", "fair", "good" or "excellent". The self-fertility of the selfed racemes was determined by the number of pods set in comparison with the apparent number of stipules. Of the 270 plants which were selfed only 26 showed a combination of low self-fertility and fair to excellent pod setting on the open-fertilized portions of the plants.

Cuttings were made of these plants and they were grown in the greenhouse during the winter of 1945–46. Upon reaching bloom a further evaluation of their self-sterility was made using the toothpick method of tripping. Many of these plants did not duplicate their field records and only 8 were retained for further study. The greenhouse record of these plants on self-fertility is given in Table 1.

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TABLE 1.—PODS AND SEEDS SET ON ALFALFA PLANTS SELECTED FOR LOW SELF-FERTILITY

Plant No.	Number of flowers tripped	Number of pods set	Per cent pods set	Seeds per pod
1	84	0	0	0.0
2	72	4	5	1.0
3	97	0	0	0.0
4	136	7	5	1.0
5	58	7	12	1.3
6	74	9	12	1.3
7	29	3	10	1.3
8	86	15	18	1.4
Av.	79	—	7.1	1.2

An average of 79 flowers were tripped per plant and the average pod-setting was 7.1 per cent, ranging from 0.0 per cent for plants No. 1 and No. 3 to 18 per cent for plant No. 8. Seeds per pod, which is also a critical measurement of self-fertility, ranged from 1 to 2 seeds per pod with an average of 1.2.

MEIOTIC BEHAVIOUR OF PARENT PLANTS

A study was made of the meiotic behaviour and related pollen viability of these selections. Studies of the actual pairing relationships of the chromosomes at first metaphase are difficult to make owing to the extremely small size of the chromosomes. On the other hand it is not difficult to discover in the stages from first anaphase to pollen tetrad whether there are any irregularities such as lagging unpaired chromosomes. Table 2 summarizes the observations of this nature on these 8 plants.

TABLE 2.—MEIOTIC BEHAVIOUR AND PERCENTAGE OF NORMAL POLLEN SELECTIONS

Plant No.	Meiosis	Per cent good pollen	Anther dehiscence
1	Regular with normal tetrads	32.8	Normal
2	"	33.5	"
3	"	46.0	"
4	"	37.0	"
5	"	41.7	"
6	"	35.3	"
7	Irregular with 2 or more univalents per cell	0–10	Frequently failed to dehisce
8	Regular with normal tetrads	93.0	Normal

Plants No. 1 to No. 6 were characterized by normal meiotic behaviour and the formation of regular tetrads; but at the time of anther dehiscence, at the pointed bud stage, the percentages of normal pollen were found to be quite low, ranging from 32 to 46 per cent. Apparently factors intervene in the course of pollen development which inhibit its proper maturation. These are postulated to be of a physiological nature although under genic control. As reported in the case of tomatoes by Rick (3) they may be due to

the failure of the tapetal layer to transfer food to the developing pollen. In these 6 plants the low percentage of good pollen is probably a factor determining self-sterility although not necessarily the only one. Armstrong and White (1) observed a correlation between the proportion of good pollen in a group of related F_1 plants and pod-setting which indicated a definite segregation of a factor (s) for pollen sterility.

Plant No. 8 resembles the first 6 plants in having regular meiotic behaviour but differs from them in the high percentage of good pollen. Self-sterility in this plant appears to be due to self-incompatibility of pollen in a similar manner to that frequently reported in several *Trifolium* species. This self-incompatibility is not absolute since 18 per cent of the tripped flowers set pods.

Plant No. 7 differs in every respect from the other plants. The occurrence of lagging univalents at first anaphase was quite common affecting most of the cells. These led to irregular tetrad formation with the lagging chromosomes thrown out as micronuclei. The pollen formed must have contained largely aneuploid chromosome numbers which inhibited normal development. The percentage of good pollen never exceeded 10 per cent. The anthers in this plant frequently failed to dehisce upon tripping while all the other plants showed normal dehiscence.

CROSS-COMPATIBILITY OF PARENTS

A study was made of the cross-compatibility of the plants by making as complete a set of diallel crosses as possible keeping the data on the reciprocal crosses separate. In crossing, due to the highly sterile nature of the material, emasculation was not considered necessary but the procedure described by Bolton (2) was followed. Flowers of the two parents were alternately tripped against a toothpick.

In Table 3 the pod-setting results are given. Twenty-two of the possible 28 diallel crosses were made. An approximate unit of 60 flowers was crossed in each case. Several facts are apparent from the Table. The general average of pod-setting was not high, being only 34.3 per cent. Another fact of importance is the differential results from the male parents. Plant No. 8, which is characterized by a high percentage of good pollen, gave the best results having an average of 60.5 per cent. Plant No. 5

TABLE 3.—CROSS-COMPATIBILITY OF PARENT PLANTS AS SHOWN BY PER CENT POD-SETTING

		Male Parents								Av. as female
		1	2	3	4	5	6	7	8	
Female Parents	1	—	26	—	21	56	38	14	50	34.2
	2	15	—	16	30	56	63	—	—	36.0
	3	18	20	—	17	40	22	—	86	33.8
	4	12	25	12	—	73	35	—	72	38.2
	5	28	60	55	57	—	10	33	60	43.3
	6	20	—	12	—	55	—	—	65	38.0
	7	5	—	22	15	6	6	—	30	14.0
	8	20	—	—	—	50	54	25	—	37.3
Av. as male		16.9	32.6	23.4	38.0	48.0	32.6	24.0	60.5	34.3

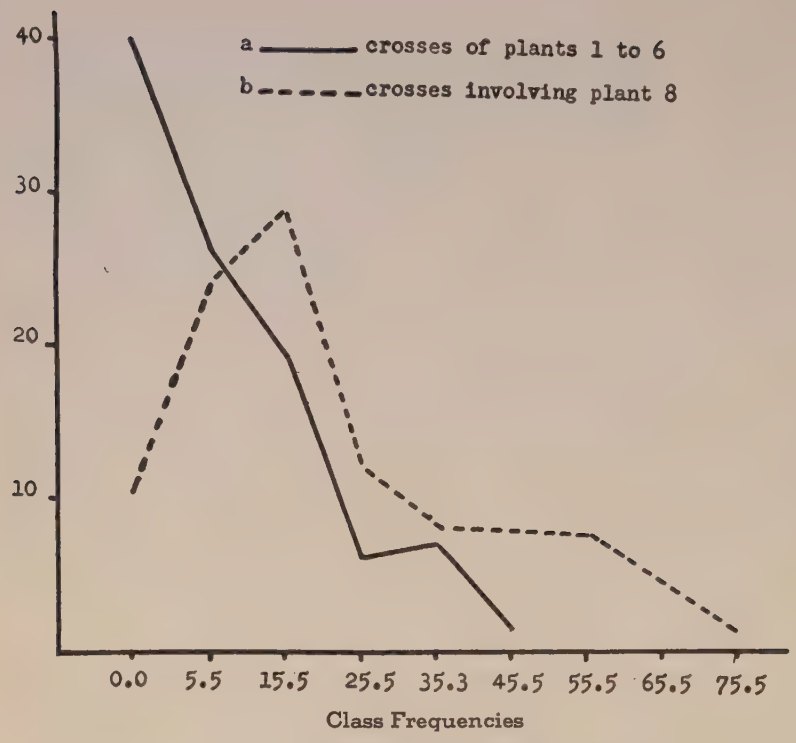


FIGURE 1. Frequency distribution of F_1 plants in regard to self-sterility; *a*, crosses of plants 1 to 6; *b*, crosses involving plant 8.

gave a fair result while plants No. 1 and No. 3 showed up poorly. It was difficult to use plant No. 7 as a pollen parent due to the scanty pollen. In the three crosses obtained with No. 7 as the male, part of the pod-setting may have been due to selfing. Rated as female parents all gave comparable results except plant No. 7 in which the 6 crosses averaged only 14 per cent. Apparently the irregular meiosis noted in the P.M.C. also characterized the M.M.C. leading to abortive embryos in a high percentage of cases.

Table 4 gives results for a similiar set of crosses in regards to seeds per pod. Here the same comparisons can be made. The average number of

TABLE 4.—CROSS-COMPATIBILITY OF PARENT PLANTS AS SHOWN BY SEEDS PER POD

		Male Parents								Av. as female
		1	2	3	4	5	6	7	8	
Female Parents	1	—	1.5	1.0	2.0	—	3.0	2.7	5.5	2.6
	2	2.1	—	1.6	1.2	—	1.5	1.5	5.8	2.3
	3	—	1.4	—	—	2.4	1.2	1.0	4.8	2.2
	4	—	—	—	—	—	1.3	1.7	4.6	2.5
	5	2.6	2.2	2.4	2.7	—	3.3	1.3	4.3	2.7
	6	1.0	—	1.3	1.0	—	—	1.6	2.7	1.5
	7	1.1	2.0	2.2	1.1	—	3.8	—	—	2.0
	8	2.5	3.1	2.7	2.0	—	3.0	2.0	—	2.6
Av. as male		1.9	2.0	1.9	1.7	2.4	2.4	1.7	4.6	2.3

TABLE 5.—DISTRIBUTION OF F_1 PROGENIES OF SELF-STERILE ALFALFA PLANTS IN PER CENT POD-SETTING

Cross	Distribution of plants in % pods set of flowers tripped									No. of plants	Av. % pods set	Seeds per pod
	0	1–10	11–20	21–50	31–40	41–50	51–60	61–70	71–80			
1 × 3	8	4								12	1.5	1.0
× 5	9	1								10	1.0	3.0
× 6	3	2	3		2					10	11.3	1.1
× 7	6	1	1		2	1				11	12.9	.9
× 8	5	2	1	2		1				11	11.1	1.1
2 × 3	3	3	3	2						11	8.3	1.2
× 4	7	2	2							11	3.4	1.0
× 5	2	3	2	1	1	1				10	19.3	.8
× 6	2	3	2	3	1	1				12	17.1	1.3
× 7	1	4	2	1		1	1			11	15.6	1.4
× 8		4	3	1		2	1			11	18.7	1.2
3 × 5	5	2	2		2					11	13.5	1.2
× 6	1	5	4	1						11	11.5	1.4
× 8	1	2	4		2	1	1			11	18.4	1.3
4 × 5	7	3	2							12	5.0	1.1
× 6	4	3	1							8	3.5	1.3
× 7	1	7	2							10	4.8	1.1
× 8	1	3	4	2	1					11	12.0	1.0
5 × 6	1	2	4	1	4					12	21.0	1.2
× 7	1	3	2							6	11.0	.8
× 8		2	3	1	2	1	3			12	25.0	1.1
6 × 7	1		4	3	2					10	21.0	1.3
× 8		3	4	2					1	10	30.0	.8
7 × 8	1		1	2	1					5	19.0	1.0
Average											13.2	1.2

seeds per pod is not high, being only 2.3 seeds. Where plant No. 8 is the male parent the seeds per pod are quite high averaging 4.6 showing that an abundance of good pollen in the stigma ensures more embryos in the ovary being fertilized. Plant No. 7 as a female did not show quite the same results as in the pod-setting study. After the initial elimination of ovaries incapable of being fertilized, the remainder contained 2 or 3 embryos which after fertilization are capable of developing into seeds.

STERILITY OF F_1 PARENTS

It is of some importance to know whether F_1 progenies from crosses of highly sterile plants would inherit this sterility or whether self-fertility would be restored as the result of hybrid vigour or as the result of bringing together complementary factors which condition sterility.

Using the seed from the diallel crosses of the parents, F_1 progenies of 10–12 plants were grown in the greenhouse in 1947–8 and their self-sterility determined. Table 5 gives the result of this study. In general it may be seen that the self-sterility of the parents is carried over into the progenies. Whereas the parents had an average of 7.1 per cent pod-setting the progenies had an average of 13.2 which is still quite low. While the seeds per pod for the parents was 1.2 the average for the progenies was also 1.2.

There is evidence, in considering the crosses involving plant No. 8, that the fertility has been raised, as if sterility was restored by bringing together complementary factors, one factor common to plants No. 1 to No. 6, and the other to plant No. 8. Omitting plant No. 7, if these crosses are totalled the two distributions are as given in Table 6.

TABLE 6.—FREQUENCY DISTRIBUTION OF F_1 PLANTS IN REGARD TO SELF-STERILITY

	Distribution in per cent pods set of flowers tripped										
	0	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	Total	Mean
8											
Others	7	16	19	8	5	5	5		1	66	19.9±2.25
%	10.6	24.2	28.8	12.1	7.6	7.6	7.6		1.5	100	
Others	52	33	25	8	10	2				1.30	7.1±1.24
%	40.0	25.4	19.2	6.2	7.7	1.5				100	

$$M_1 = 19.9 \pm 2.25 \qquad M_2 = 7.1 \pm 1.24$$
$$M_1 - M_2 = 11.8$$

$$\sigma m_1 - m_2 = \sqrt{2.25^2 + 1.24^2} = 2.57$$

$$t = \frac{11.8}{2.57}\sqrt{\frac{130 \times 66}{196}} = 30.36$$

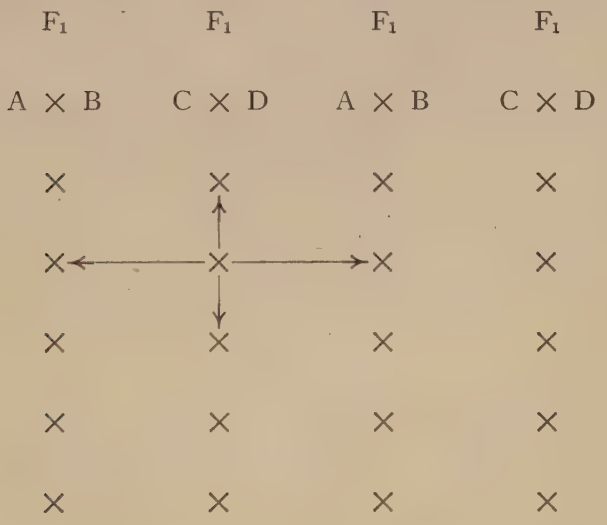
The t value is highly significant.

These results may be interpreted to mean that plant No. 8 and the plant group No. 1 to No. 6 owe their self-sterility to different factors. These factors probably operate in a manner indicated by their cytological behaviour. One factor affects the proportion of good pollen while the other affects the self-compatibility of the pollen. It is logical to expect that each of these factors has a range of allelomorphic factors and also modifying factors. This is suggested by the range of distributions in the crosses from the plants in the 1–6 group. The significant fact of this study is that fertility will be restored in part in the F_1 generation upon crossing two highly sterile parents if they differ in sterility factors and conversely, sterility will be retained in F_1 if the sterility in the parents is conditioned by the same factor or type factor.

CROSS-FERTILITY IN THE F_1 GENERATION

Theories on the production of hybrid alfalfa in commercial quantities require the use of only four superior plants. Each F_1 generation of seed can be obtained by using alternate clonal rows of two selected parents in an isolated nursery. The high self-sterility of each parent ensures that the seed produced will be the result of out-crossing and hence possess the maximum expression of hybrid vigour. To produce the second generation these two F_1 lots of seed can be mixed in equal amounts and sown by growers in good seed producing areas to produce the hybrid seed of commerce. To retain or augment the vigour of the F_1 generation is vital, and this would depend upon the degree of crossing between related and unrelated F_1 plants.

The two lots of F_1 plants may be visualized in rows as follows:



There is the same opportunity for a plant to cross with a sister plant as with an unrelated plant from the other F_1. Presumably the pollinating bees visiting and tripping the flowers will be primed with both types of pollen. If both types of pollen are equally viable on the stigmas of a

TABLE 7.—CROSSING RESULTS OF RELATED AND UNRELATED F_1 PLANTS PER CENT PODS SET OF FLOWERS CROSSED

Parent Cross	Progeny No.	3–2–5	3–2–6	3–2–7	3–2–8	5 × 7			
						2–9–1	2–9–3	2–9–4	2–9–5
1 × 3	3–2–5		8			67	20		
	–6	4		0	0	68	62		
	–7	5	0					59	16
	–8		2	7				66	30
		Av. = 3.2%				Av. = 48.5%			
5 × 7	2–9–1	26	5				4		0
	–3	62	12			5		12	
	–4			2	8		4		5
	–5			2	16	0		0	
		Av. = 16.7%				Av. = 3.7%			

TABLE 8.—CROSSING RESULTS OF RELATED AND UNRELATED F_1 PLANTS PER CENT PODS SET OF FLOWERS CROSSED

Cross	Plant No.	2–6–1	2–6–2	2–6–3	2–6–4	1–4–1	1–4–3	1–4–4	1–4–5
2 × 6	2–6–1		2	0		70	38		
	–2	0			0	18	58		
	–3	0						14	74
	–4		10					52	56
		Av. = 2.0%0				Av. = 47.5%			
4 × 8	1–4–2	6	8				4		17
	–4	20	3					5	3
	–4			30	5		26		10
	–5			21	4				6
		Av. = 12.1%				Av. = 10.1%			

given plant there would be an equal amount of inbred and out-crossed seed and the whole aim of breeding hybrid alfalfa to secure maximum vigour would be defeated.

To answer this question a further set of crosses were made within the F_1 lines and the likelihood of related and unrelated plants crossing, interpreted in terms of the percentage of pod-setting obtained. Tables 7 and 8 give the results of this test. In Table 7 four F_1 plants from the cross 1 × 3 were crossed among themselves (8 crosses) and also with 4 F_1 from the cross 5 × 7 (8 crosses). The difference is quite striking. Crosses between related plants averaged only 3.2 per cent pods set while the crosses between unrelated plants averaged 48.5 per cent. Although no counts were made of seeds set per pod in these crosses, an estimate indicated the outcrosses had 4 to 6 seeds per pod while the inbreds had 1 to 2. In this case there is

little doubt that under field conditions the seed produced on each F_1 plant would be largely the result of out-crossing. In the reciprocal crosses given in the same table, the crosses between related plants in the 5 × 7 F_1 gave 3.7 per cent pod-setting. The results for the out-crossing is not quite as good as in its reciprocal. This may be the result of plant No. 7 producing faulty megaspore development.

Table 8 shows a similar set of crosses using two different F_1 lines, 2 × 6 and 4 × 8. Crosses between F_1 plants within the F_1 line 2 × 6 averaged only 2.0 per cent pod-setting while out-crosses with F_1 plants from 4 × 8 averaged 47.5 per cent. These results are comparable to those given in the previous Table. However the inbred crosses in the 4 × 8 F_1 line showed different results having an average of 10.1 per cent which was almost equal to its out-cross at 12.1 per cent. There is a definite indication of higher compatibility in related F_1 plants in this cross than in the other three tested. This is probably due to the partially restored fertility in F_1 involving (1–6) × 8. The result is important in considering how selected self-steriles should be combined to produce the final double-cross seed.

CONCLUSIONS

Highly self-sterile alfalfa plants may owe their sterility to the operation of several genetic factors. In the present study three distinct types of sterility have been encountered. The sterility type due to meiotic irregularity should be avoided as plants of this type are both poor pollen and seed parents and may perpetuate an unstable chromosome condition in the hybrids. Sterility due to physiological disturbances in the maturing of the pollen is a second type and such plants are useful in the progress as they may prove to be valuable combiners. The third type of sterility, that due to self-incompatibility, is very desirable as such plants produce abundant pollen.

In designing the crossing there should be available two superior plants of type two and two from type three. The F_1 generations should be within the types and not across the types in order that self-sterility will be retained and crossing between related F_1 plants reduced to a minimum. In this way the double-cross seed will be almost entirely the result of outcrossing when the two different F_1 lines are brought together.

The identification of these different sterility types reported in this paper indicates the need of a careful cytological study of selected self-steriles before they are used in a breeding program.

SUMMARY

An examination of highly self-sterile alfalfa plants showed that self-sterility might be due to (1) meiotic irregularity; (2) a high percentage of poor pollen produced by faulty maturation, and (3) self-incompatibility of pollen in plants which produced a high percentage of good pollen.

Crosses within these classes lead to certain conclusions. Plants characterized by meiotic irregularity are both poor male and female parents and should be avoided in a breeding program. Crosses within the second class of self-steriles produced progenies in which the average self-sterility

was very similar to that of the parents. Crosses between the second and third classes of steriles produced progenies in which self-sterility was restored to a significant degree, presumably due to the operation of complementary factors.

In designing a breeding program to produce hybrid alfalfa by utilizing highly self-sterile plants, the breeder should have sufficient plants of type two and type three so that the first generation crosses can be within the types. Later two first generations tracing to the two different types can be combined under field conditions and the maximum vigour due to complete outcrossing will be ensured to the resulting hybrid.

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THE NITROGEN DISTRIBUTION AND AMINO ACID CONTENT OF CERTAIN SOIL ORGANIC MATTER FRACTIONS[1]

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It has long been held that a considerable proportion of the soil nitrogen is combined in the form of protein; about one-third of it is liberated as amino-N on acid hydrolysis. Recently Kojima (11) made a quantitative estimation of the amount of aspartic acid, glutamic acid, leucine, isoleucine, valine and the hydroxyamino acids in a muck soil. Bremner (5), using the technique of paper partition chromatography identified, in addition to the above acids, phenylalanine, alanine, glycine, lysine, arginine, histidine, proline, hydroxyproline, α, ϵ-diaminopimelic acid, α-amino-n-butyric acid and tyrosine in the hydrolysates of a number of soils. Other workers (14, 17) have also isolated amino acids from soil hydrolysates.

In a previous publication (8) a paper partition chromatographic technique for the identification and quantitative estimation of the amino acids in soil organic matter fractions was described. This technique was used in the present investigation to study the nitrogen distribution and amino acid composition of three soils and of the organic fractions isolated from them. The purpose of the study was to determine whether or not the nitrogen-containing materials in the different soils and in their various fractions are similar.

EXPERIMENTAL

Nature of soils and organic fractions

The three soils chosen for the investigation were a podsol from Lennoxville, Quebec, a dark brown prairie soil from Scott, Saskatchewan, and a black soil from Lacombe, Alberta.

The humate fractions were isolated from the sodium-saturated soils by a method similar to that described by Atkinson and Turner (2). They were precipitated with acid at pH 2, dried in a vacuum oven at 50° C. and ground to pass a 60-mesh screen. The soils and the organic matter fractions isolated from them are more fully described elsewhere (16).

Hydrolysis

The samples were hydrolysed under reflux for 22 to 24 hours with 20 ml. of 6 N HCl per gram of material.

Methods of nitrogen analysis

Total-N was determined by the Kjeldahl method using the digestion mixture of Campbell and Hanna (6). Ammonia-N and amino-N were determined by methods described by Van Slyke (18, 19).

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Nitrogen distribution

The acid hydrolysate was centrifuged and the residue washed with water until the washings were colourless. The residue was dried, weighed, ground to pass an 80-mesh screen and analysed for insoluble nitrogen. Ammonia-N and total soluble-N were determined on aliquots of the combined supernatant liquid and washings. The material left after removal of the ammonia-N was centrifuged and the $Ca(OH)_2$ precipitate washed with small amounts of water until the supernatant liquid and washings reached a volume of 25 ml. Humin-N was determined on the $Ca(OH)_2$ precipitate and amino-N on the supernatant liquid.

Paper-partition chromatography.

One- and two-dimensional chromatograms were used to identify the amino acids while one-dimensional chromatograms, in phenol and o-cresol, were used to estimate the amounts present.

The amount of hydrolysate placed on the paper was increased by applying up to six successive drops of 0.005 to 0.015 ml. to the same spot, letting each drop dry thoroughly before applying the next. In this way, smaller, less diffuse and more highly coloured spots were obtained on spraying the paper after separation of the acids. The higher amino acid concentration also increased the colorimeter readings for the quantitative determination.

RESULTS AND DISCUSSION

Nitrogen distribution

The nitrogen distribution in the original soils and in the humate fractions separated from them are shown in Table 1. The data indicate that there were no distinctive differences in the nitrogen distribution either

TABLE 1.—NITROGEN DISTRIBUTION IN THE SOILS
(as % of total N)

—	Soil	Group 1 Colloids		Group 2a Colloids		Group 2b Colloids
		1HF*	2HF*	1HF*	2 HF*	1HF*
Lacombe Soil—						
Insoluble N	31.9	38.6	41.4	33.6	34.5	31.9
Soluble N	73.7	43.2	53.0	53.5	64.8	66.8
NH_3—N	21.9	15.9	14.4	15.9	14.5	18.3
NH_2—N	13.9	12.5	18.8	15.5	20.6	27.2
Humin—N	14.2	5.1	5.6	8.8	7.7	9.6
Scott Soil—						
Insoluble N	16.7	33.1	32.2	23.1	33.3	11.9
Soluble N	87.6	60.7	71.5	68.5	71.6	74.0
NH_3—N	18.7	17.2	14.5	18.5	17.8	16.2
NH_2—N	18.7	18.6	25.4	27.2	19.7	39.6
Humin—N	18.7	11.0	8.9	13.9	13.5	14.6
Lennoxville Soil—						
Insoluble N	19.8	19.3	37.7	22.8	24.7	21.2
Soluble N	83.9	65.1	60.3	71.4	74.2	68.7
NH_3—N	24.2	14.1	13.0	17.8	17.3	13.5
NH_2—N	19.8	27.2	24.2	26.6	29.9	23.6
Humin—N	24.2	8.6	14.6	2.5	13.7	12.0

* HF = Humate Fraction.

between soils or between fractions of each soil. It was found that 43 to 74 per cent of the nitrogen of the humate fractions was made soluble on hydrolysis and that from 12 to 41 per cent of the nitrogen remained in the residues. More nitrogen was liberated on hydrolysing the original soils, 74 per cent for the Lacombe soil, 84 per cent for the Lennoxville and 88 per cent for the Scott soil, with 32, 20 and 17 per cent respectively left in the residues. The soluble nitrogen of the Lacombe soil and its organic fractions was slightly lower than for the other soils and the organic materials isolated from them; its insoluble nitrogen was higher. In general, the percentage of insoluble nitrogen was lower in the soils than in their organic fractions; this was to be expected since a large amount of the more soluble nitrogen was lost during the isolation of the humate fractions.

The ammonia-N accounted for 13 to 24 per cent of the total nitrogen with a slightly larger amount present in the soils than in the fractions. The amino-N accounted for 13 to 40 per cent of the total nitrogen. In each of the three soils the amino-N of the original soil was somewhat lower than that of its organic fractions. The Lennoxville fractions had a relatively higher amino-N content than those of the other soils. There was relatively less humin-N in the Lacombe soil and the fractions from it.

The nitrogen distribution of the soils and fractions investigated was similar to that reported by other workers (4, 10, 13) who found that 66 to 86 per cent of the nitrogen was liberated by hydrolysis. With the exception of some of the Lacombe soil fractions, the results reported here are not greatly different. Kojima (10) reported that 37 per cent of the total nitrogen of a muck soil was amino-N and Bremner (4) found that 24 to 36 per cent of the nitrogen of the soils he investigated was amino-N. In the present investigation it was found that the amino-N content was, on the whole, somewhat lower.

TABLE 2.—AMINO ACID DISTRIBUTION IN THE LENNOXVILLE (PODSOL) SOIL
(Mgm./gm. of fraction, ash-free basis)

——	Soil	Group 1 Colloids		Group 2a Colloids		Group 2b Colloids
		1HF*	2HF*	1HF*	2HF*	1HF*
Aspartic acid	+	6.0	4.4	12.2	18.2	6.7
Glutamic acid	+	7.7	5.6	7.0	14.3	10.5
Serine	+	6.9	6.0	—	—	5.7
Glycine	+	3.5	4.9	10.7	16.0	4.8
Threonine	+	4.3	6.5	13.3	12.7	8.4
α-alanine	+	—	10.9	—	—	—
Tyrosine	—	4.9	—	13.7	—	—
β-alanine	+	6.8	11.6	—	—	15.7
Histidine	—	—	18.5	—	—	—
Lysine	+	—	11.8	13.3	17.4	9.6
Arginine	—	—	—	—	—	—
Valine	+	4.0	11.2	10.6	13.5	5.4
Leucine	+	2.9	11.1	7.8	13.0	7.7
Proline	+	—	Trace	—	Trace	—
Hydroxyproline	—	—	—	Trace	—	—
Phenylalanine	—	—	—	—	—	7.7
"Under serine"	+	—	—	—	—	—

* HF = Humate Fraction.
\+ Indicates amino acid present.
— Indicates amino acid absent.

TABLE 3.—AMINO ACID DISTRIBUTION IN THE SCOTT (DARK BROWN PRAIRIE) SOIL (mgm./gm. of fraction, ash-free basis)

—	Soil	Group 1 Colloids		Group 2a Colloids		Group 2b Colloids
		1HF*	2 HF*	1 HF*	2HF*	1HF*
Aspartic acid	+	4.7	4.3	8.2	—	6.9
Glutamic acid	+	6.5	5.1	7.9	2.4	7.6
Serine	+	4.9	7.1	7.3	9.6	8.6
Glycine	+	3.9	4.6	4.1	3.6	7.6
Threonine	+	6.5	7.9	8.7	5.5	12.3
α-alanine	+	—	4.1	6.2	—	20.6
Tyrosine	—	—	—	—	7.7	—
β-alanine	+	4.9	8.1	—	6.7	27.0
Histidine	+	—	6.8	15.2	—	—
Lysine	+	6.2	9.0	—	2.4	19.6
Arginine	—	—	—	—	2.4	—
Valine	+	3.9	5.5	4.1	4.6	23.6
Leucine	+	4.4	5.7	6.8	2.9	11.3
Proline	+	Trace	—	Trace	—	—
Hydroxyproline	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—
"Under serine"	—	4.9	6.5	—	—	—

* HF = Humate Fraction.
\+ Indicates amino acid present.
— Indicates amino acid absent.

TABLE 4.—AMINO ACID DISTRIBUTION IN THE LACOMBE (BLACK) SOIL (mgm./gm. of fraction, ash-free basis)

—	Soil	Group 1 Colloids		Group 2a Colloids		Group 2b Colloids
		1HF*	2HF*	1HF*	2HF*	1HF*
Aspartic acid	+	4.1	4.7	4.4	3.9	4.0
Glutamic acid	+	5.9	5.2	5.4	6.7	5.6
Serine	+	3.5	4.3	4.2	5.6	8.1
Glycine	+	3.3	4.0	2.4	4.5	4.2
Threonine	+	5.1	4.7	5.4	6.1	6.8
α-alanine	+	5.5	5.4	4.0	3.2	6.3
Tyrosine	—	—	4.3	—	6.4	—
β-alanine	+	4.9	5.0	—	6.4	6.4
Histidine	+	3.1	—	—	—	—
Lysine	+	2.9	6.7	5.8	6.9	7.6
Arginine	—	—	Trace	—	—	8.5
Valine	+	3.1	5.4	6.8	7.5	6.8
Leucine	+	2.5	3.2	5.0	4.7	9.3
Proline	+	—	Trace	Trace	Trace	Trace
Hydroxyproline	—	—	—	—	—	Trace
Phenylalanine	—	—	—	—	—	6.4
"Under serine"	+	—	4.3	—	8.8	—

* HF = Humate Fraction.
\+ Indicates amino acid present.
— Indicates amino acid absent.

Amino acid composition

The amino acid composition of the soils and organic matter fractions is reported in Tables 2, 3 and 4. The quantitative determinations were made on at least five separate spots containing varying amounts of the amino acid and the results reported are averages of these determinations. Qualitative determinations only were made on the hydrolysates of the three soils as the spots were too diffuse and streaky for accurate analysis. This was perhaps due to the presence of salts in the hydrolysate.

There were no distinctive differences in the amino acid composition either between soils or between the fractions isolated from each soil. No direct comparison of the distribution in the soils and their fractions could be made since some of the amino acids may have been lost in the isolation of the colloids. Also, an amino acid present in a small amount in a small fraction only may not be identified in the soil as a whole. The amino acids identified were somewhat similar to those reported by Bremner (5). Some of the acids he found were not present in any of the soils investigated although most were detected in some of the fractions.

Glutamic acid, glycine, threonine, valine and leucine were found in all the hydrolysates of the soils and their fractions. They occurred in relatively higher concentration in the Lennoxville fractions than in those from the Lacombe soil or the Scott soil. It has been suggested (5) that a large part of the glycine found in soils is derived from the decomposition of nucleic acids under the conditions of acid hydrolysis.

Aspartic acid, lysine and serine were detected in the three soils and in nearly all the fractions. Aspartic acid and lysine were found in a relatively larger amount in the Lennoxville fractions while the Scott fractions contained more serine. α- and β-alanine, found in slightly larger amounts in the fractions of the Scott soil, were detected in approximately two-thirds of the humate fractions and in the original soils. Proline and hydroxyproline, when present, were found in traces only and were not determined quantitatively. Proline was found in the hydrolysates of the original soils but hydroxyproline could be detected only in some of the humates. Tyrosine and phenylalanine were found in a few of the fractions but were absent from the soil hydrolysates.

Histidine and arginine were found in only a few of the fractions with histidine being detected more often and in greater amounts than arginine. Arginine was not found in the hydrolysates of any of the three original soils, while histidine was found in the Lacombe and Scott soils. Schreiner and Shorey (15) isolated these two amino acids from a Houston soil by alkaline extraction at room temperature. They reported that in a number of other soils examined histidine was found more often, and in greater amounts, than arginine. Bremner (5) identified arginine in all the soils he examined. It is possible that most of the arginine and histidine was lost in the filtrates of the fractions during their isolation. However, this does not seem likely in view of the fact that arginine was not found in the original soils and that both acids were detected more frequently in the alkaline-extracted fractions than in the water-extracted ones. Chibnall (7) has noted the destruction of large amounts of some of the amino acids of plant proteins during acid hydrolysis in the presence of carbohydrate material. While it is possible that this may account for some of the loss of amino acids (e.g., by a condensation of amino acids with polyuronide decomposition products) it could hardly account for the fact that very little arginine is recovered from soil organic matter. Lea and Hannan (12) have reported that arginine and, to a lesser extent, lysine and histidine could not be fully recovered in the acid hydrolysate of the "browning reaction" product of casein and glucose. The fact that arginine and histidine were found in only small amounts in the hydrolysates of soil organic material may indicate that these acids are destroyed by a reaction of this type during the course of formation of soil organic matter.

Cystine and methionine, the two sulphur-containing amino acids, were not found in the hydrolysates of any of the materials studied. Bremner (5) also failed to detect these acids in soils and suggests that they may be decomposed by secondary reactions during acid hydrolysis. There are traces of organic sulphur found in soils and some of it is presumed to be present in the form of sulphur-containing amino acids. No organic sulphur determinations were made on the soil or fractions in this investigation.

A pink-purple spot was located between glutamic acid and serine with an R_f value of 0.28 to 0.31 when run in phenol; this spot is referred to as "under serine" in the tables. In a map of spots published by Bremner (5) α, ϵ-diaminopimelic acid was shown to take a position similar to this. Bremner reported that this amino acid was present in each of the 10 soils he investigated in relatively small amounts. Since no pure α, ϵ-diaminopimelic acid was available it was not possible to confirm the identity of this spot. This compound could not be detected in the Lennoxville soil fractions or the original Scott soil hydrolysate. For the quantitative determination of this material the factor (8) for converting reading to amount present was arbitrarily chosen at 5.0 and its amino-N content was assumed to be 10 per cent.

When the amino acid composition of the soils, and more especially of the fractions isolated from them, is compared with that of the proteins of leaves, grasses and microorganisms, some striking differences are evident. Typical analyses of these materials (1, 7, 9) show the presence of large amounts of arginine with relatively smaller amounts of histidine and tyrosine. In the soil material this relationship is reversed. It does not seem likely that arginine would be lost or destroyed during hydrolysis to a large enough extent to account for this difference. Hence it would seem that if unaltered plant or bacterial proteins are combined in soil organic matter, that combination must be of such a nature that arginine is destroyed preferentially during hydrolysis. It would seem more probable, however, that the protein itself is altered during humus formation. The absence of cystine and methionine from the soil organic matter fractions is also difficult to explain on the assumption that bacterial or plant proteins combine directly with lignin to form humus.

The per cent of total amino nitrogen recovered as amino acid nitrogen is recorded in Table 5. While the amounts showed considerable variation, in general a large percentage of the amino-N was accounted for in this way.

TABLE 5.—RECOVERY OF AMINO NITROGEN

(N as % of total NH_2—N)

——	Lacombe Soil	Scott Soil	Lennoxville Soil
1 HF*, gp 1	124	97	54
2 HF*, gp 1	89	82	89
1 HF*, gp 2a	81	62	107
2 HF*, gp 2a	83	60	108
1 HF*, gp 2b	92	102	117

*HF=Humate Fraction.

In general the amino acid composition and nitrogen distribution data do not indicate that the organic matter has been separated into chemically different fractions nor do they offer any explanation for the claim (3) that the group 1 fractions, especially the 1st humate, are more important from a soil fertility standpoint than are the group 2 fractions.

SUMMARY

The nitrogen distribution and amino acid composition of organic matter fractions isolated from three different soils (a podsol, a black soil and a dark brown prairie soil) have been studied. The fractions were peptized with water and dilute alkali from the sodium-saturated soil and precipitated with acid. No distinctive differences in the Van Slyke nitrogen distribution were noted either between soils or between different fractions of the same soil. The amino acids were determined qualitatively and semiquantitatively by paper chromatography. Again there were no sharp differences in the amino acid content of the various soil organic matter fractions. Glutamic acid, glycine, threonine, valine and leucine were found in all the fractions, aspartic acid, serine and lysine in nearly all, α- and β-alanine in about two thirds, and the others—histidine, proline, hydroxyproline, arginine, phenylalanine—in less than one third of the fractions. The fractions differed from plant proteins in that no sulphur-containing amino acid and very little arginine and histidine were found.

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A NOTE ON THE DORMANCY OF TARTARY BUCKWHEAT SEEDS

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Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is a prolific seed producer. During the growing season, flowers, immature and mature seeds (achenes) may be found on the same inflorescence at the same time. The seed is not normally germinable at the time of shedding and the behaviour of the plant is decidedly weedy in all respects.

In September 1942, at the request of the Alberta Department of Agriculture, the author made a microscopic examination of the seeds and conducted a few trial experiments on their germinability. Unfortunately the project was terminated at an early stage but, because of renewed interest in this weed, a number of observations made at that time are worthy of recording now.

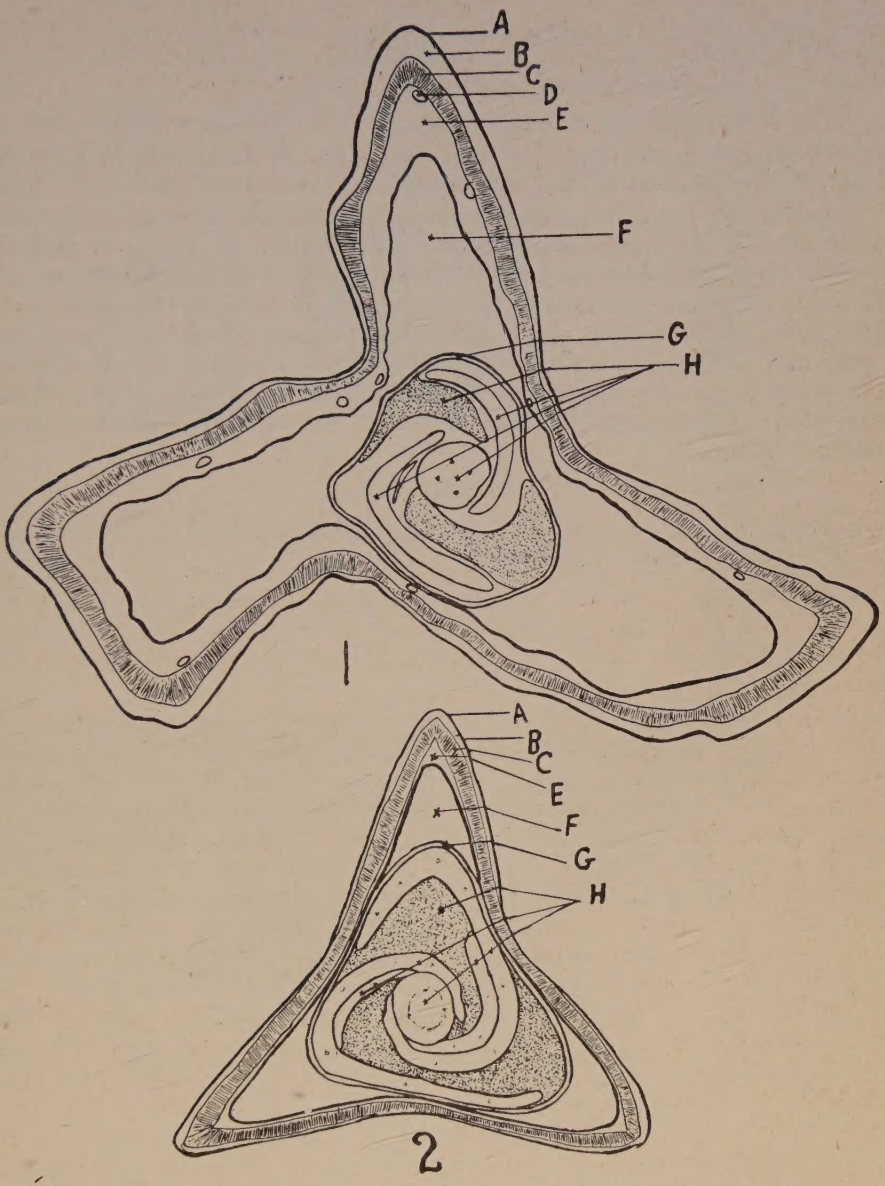
On September 10, 1942, a large quantity of seed in various stages of maturity was collected from badly infested fields in the Edmonton district. Some of this seed was fixed immediately in formalin-aceto-alcohol. Some time later, the seeds were dehydrated, embedded in paraffin and finally sectioned transversely by microtome at a thickness of 12μ. Since the embryo appeared to be well developed at maturity, the microscopic examination of transverse sections was directed to the seed coats and pericarps of immature and mature seeds.

In a green immature seed (Figure 1) the seed coat consists of a single row of large cells whose walls give a positive test for cutin. The pericarp, on the other hand, is made up of three distinct layers: (1) an outer layer consisting of a single row of extremely large and heavily cutinized epidermal cells; (2) a middle layer of sclerenchyma fibres; and (3) a wide inner layer of parenchymatous tissue. Actually the middle layer is a double layer since it consists of an outer band of three or four rows of horizontal fibres and an inner band of one, two or three rows of vertical fibres. This fibrous layer is continuous around the whole pericarp except at the apical end where it is broken at the corners by patches of parenchyma.

With increasing maturity the pericarp turns a greyish brown colour and the epidermal and parenchymatous layers become considerably shrunken and ruptured (Figure 2). Progressive thickening and lignification of the fibre walls occur until at maturity the pericarp becomes so hard that it is almost impossible to cut. The cells of the seed coat become greatly compressed and their walls so heavily cutinized that, by the time the seeds are mature, this tissue appears to be completely impervious to water. The enclosed embryo is fully developed at this stage.

In regard to the germinability of tartary buckwheat seeds Lewis (1) found that they are not germinable when shed, but that they may be roused from dormancy by drying. The results of a few germination tests in the present study may be briefly summarized as follows:

(1) Plump, uniformly mature seeds are not germinable at the time of shedding. Seeds dried for a period of 10 to 30 days at 25° C., prior to germination, germinated poorly and spasmodically over a two-week period,



FIGURES 1 AND 2. Cross sections of immature and mature seeds (× 15).

Pericarp:
- A—cuticle
- B—epidermis
- C—sclerenchyma fibres
- D—vein
- E—parenchyma

F—space separating pericarp and seed
G—seed coat
H—embryo and endosperm

after which time no germination took place. Seed dried for a period of 60 to 70 days prior to germination germinated 100 per cent within two to three days, thus confirming Lewis's observations.

(2) Seeds kept under damp conditions up to 60 days from the time of shedding failed to germinate. After a drying period that varied in duration from 30 to 70 days, at 25° C., germination increased from 0 to 100 per cent.

(3) In the majority of cases, seeds that remained dormant under the above conditions showed good germination within 1 to 3 days after both pericarps and seed coats had been removed. Seeds with pericarps removed, but with seed coats still intact, did not germinate; but, once the seed coats were also removed from these same seeds, approximately 100 per cent germination took place within one to three days. That dormancy is broken immediately on the removal of the seed coat in these seeds is highly significant and suggests the possibilities of further investigations along these lines.

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